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THE EFFECT OF MODULATED HIGH FREQUENCY CONDENSER FIELD ON THE STRAUB-FUEHNER FROG HEART PREPARATION

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Additional interest in Physical Medicine has resulted from the publication of a recent report (1). Medical diathermy is recognized by many as a field of endeavor very incompletely investigated. No particular effort has been made to ascertain the possible effects of amplitude and frequency modulated currents upon living matter. In respect to the usual diathermy the general tendency has been to explain all physiologic effects as due to thermal changes, little or no effect is ascribed to specific or selective mechanical, chemical or thermal action. Thus capillary pressures, fluid interchanges, conductivities, blood states, metabolism, etc., are said to be modified directly or indirectly as the result of the physiological responses normally following the production of heat. Specific or selective heating and specific or selective biologic action of high frequency current remain unproven and subject to question (2).

It is the desire of the authors to bring to the attention of those interested the following observations which apparently demonstrate a selective action of modulated high frequency current which is associated with modification of the normal function of the excised frog heart preparation (3). In this study the preparation is suspended in place between the plates of an auxiliary tuned circuit consisting of glass-plate-air and inductance (4). The preparation is thus subject to a controllable modulated high frequency condenser field (fig. 1). This tuned circuit is placed in proper coupling relationship to the tank circuit of a self controlled oscillator operating at approximately 125 megacycles and modulated at approximately 1000 cycles (5). The Radio Frequency output of this combination is approximately 4 watts.

Figure 2, A, B, C, D, E, F, illustrates the manner in which the field effects the spontaneous rythmical activity of various excised heart preparations. It is apparent from these exhibits that there results either cardiac or ventricular standstill. In all instances the standstill shown was temporary and reversible. Furthermore, preliminary changes in rate and amplitude may occur in advance of

standstill. A shows no change in rate and amplitude, B shows predominantly a decrease in amplitude, C shows preliminary decrease in amplitude with sub-

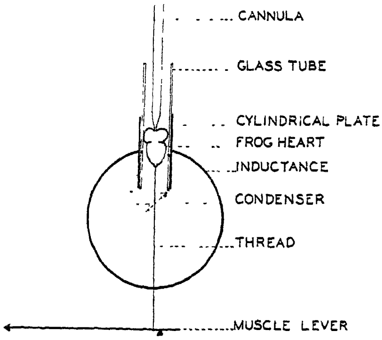

Fig. 1

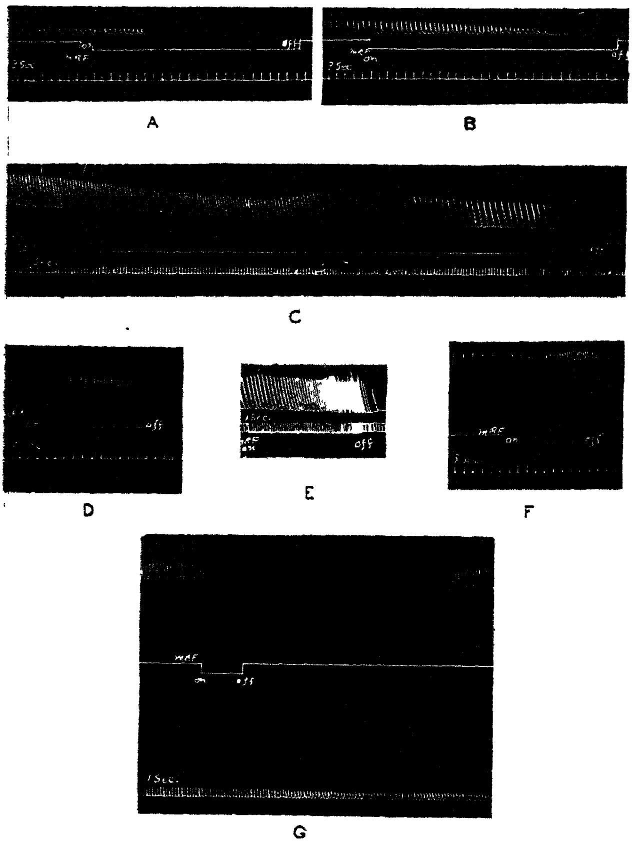

Fig 2

sequent slowing and changes in amplitude, D shows acceleration then slowing associated with decreased amplitude, E shows acceleration, F shows acceler-

ation and rise in diastolic tone, G shows initial ventricular failure followed by auricular failure with subsequent recovery in the reverse order.

The consistent feature of all these recordings is the ultimate cardiac standstill. Some preparations show initial effects pointing to block of ventricular function, the majority show initial changes indicative of blocked functional conduction superior to the atria. The evident failure of all preparations to respond in the same manner previous to standstill is probably due to such factors as inherent individual characteristics, size of heart, position of heart relative to plates, amount of leak, coupling to oscillator, tuning of auxiliary circuit and percent and type of modulation.

The observation has been made that with a given preparation it is possible accurately to reproduce a particular effect if such is the desire by controlling, for example, the exposure time. Again we have evidence of the induction of intermittence of action which is prone to follow longer exposures. Intermittency has persisted as long as two and a half hours and would have continued longer. Irrigation and bathing the preparation with fresh Ringer's was sufficient to restore normal rhythmical activity. The intermittent activity present resembles the type of ventricular action shown by Sollman and Barlow (6) to be due to epinephrine action. However, the intermittency is that of the intact heart rather than ventricular strip.

Electrographic studies were made of preparations during the application of the modulated high frequency current. The leading employed was as follows: right arm lead to the fluid in the cannula, left leg lead to the tip of the ventricle. Our observations indicate abrupt cessation of action potentials was associated with mechanical standstill. During recovery the electrical and mechanical manifestations usually come on simultaneously; however, we have on occasion observed preliminary action potentials not associated with mechanical response.

It is important to point out that prolonged exposures were associated with manifestations of irreversible changes. For example, the heart may not again show spontaneous activity, either of atrial or ventricular nature. In all such instances the atrial and ventricular muscle was found to be responsive to mechanical and elctrical stimuli. With additional exposure of suitable length an apparent irreversible heat contraction of ventricular muscle associated with characteristic physical changes makes its appearance.

The question arises as to possible mechanisms acting to produce the results. From our investigation we believe that a selective action takes place on the nerve terminals, the pacemaker and the associated conduction system. That the action is not the result of a generalized increase in temperature is indicated by the fact that an equal amount of saline corresponding to the capacity of the heart and its saline contents shows approximately a 1°C. rise in temperature when exposed to the field for 30 to 40 seconds. In order to produce some similarly appearing results Mott and Fenning (7) found it necessary to subject similar preparations to the brief thermal action of Ringer's heated to 40–80°C.

A selective, relatively rapidly acting mechanism is in operation with the use of the modulated high frequency condenser field. Selective heating may account

for the results since it is known that a high frequency electrostatic field will preferentially heat oils faster than salines (8). In addition there is some evidence of chemical factors produced or activated during exposure. Transference of fluid from the heart exposed to the field to a normal control: in some instances depression and standstill resulted and in other instances there has resulted acceleration and improved action. There thus may be present in addition to selective heating two or more chemical factors having some action. This phase of the problem is undergoing additional investigation.

Conclusions. Characteristic cardiac and ventricular standstill of the Straub-Fuehner frog heart can be produced using modulated high frequency current applied in the manner shown by the authors.

The results so obtained show a selective action of the current, the specific nature of whose action is not understood.

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EXPERIMENTAL STUDIES ON MAN WITH A RESTRICTED INTAKE OF THE B VITAMINS[1]

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PART I. THE BORDERLINE OF DEFICIENCY

For several years this Laboratory has studied experimentally the relation in normal men between intake of vitamins of the B complex and various aspects of "fitness," including work performance and capacity, cardiovascular, sensory and psychomotor functions, and aspects of intermediary metabolism. Measurements of intellective and other purely psychological factors have been added as suitable methods have been devised and standardized.

Previous reports have indicated: 1, failure to discover any benefit from vitamin "supercharging" above the National Research Council recommended allowances (Keys and Henschel, 1942); 2, apparent adequacy for at least several months of 0.23 mgm. of thiamine per 1000 Cal. (Keys, Henschel, Mickelsen and Brozek, 1943); 3, apparent adequacy for at least 152 days of 0.31 mgm. of riboflavin per 1000 Cal. (Keys, Henschel, Mickelsen, Brozek and Crawford, 1944); 4, absence of deterioration in 14 days of hard physical work on a diet providing, per 1000 Cal., 0.16 mgm. of thiamine, 0.15 mgm. of riboflavin and 1.8 mgm. of niacin (Keys, Henschel, Taylor, Mickelsen and Brozek, 1944). The present paper is a report of results from studies during subsistence for 161 days on a basal diet providing an average, per 1000 Cal., of 0.185 mgm. of thiamine, 0.287 mgm. of riboflavin and 3.71 mgm. niacin, with other members of the B complex more or less in proportion. Caloric balance was maintained on a diet providing an average of 3300 Cal. per day.

The general program and procedures were similar to those used previously (cf. op. cit.), with modifications to increase the rigidity of control and the sensitivity and completeness of the test methods. All subjects were studied in an extended preliminary standardization and control period. The terminal control consisted of a 33-day experiment with a synthetic diet providing substantially no vitamins of the B complex; the extensive results from this final experiment are presented in a separate section.

[1] This work was supported in part under the terms of a contract, recommended by the Committee on Medical Research, between the Regents of the University and the Office of Scientific Research and Development. Important financial assistance was also provided by the Nutrition Foundation, Inc., the U. S. Cane Sugar Refiners' Association, N. Y., the Corn Industries Research Foundation, N. Y., Swift and Co., Chicago, the National Confectioners' Association, Chicago, the National Dairy Council, Chicago, and the Graduate Medical Research Fund, University of Minnesota. Merck and Co., Inc., provided a generous supply of pure vitamins. Most of the food materials were supplied by the Subsistence Branch, Office of the Quartermaster General, U. S. Army.

Subjects. The subjects were 8 conscientious objectors transferred from Civilian Public Service Camps and resident in this laboratory throughout the experiment. They were selected from volunteers and were free from significant organic disease and defects as well as from significant psychopathology. Some of their characteristics are listed in table 1. They had previously been of sedentary to moderately athletic habits. Prior to transfer all had subsisted for some months in C.P.S. camps where the diet had been cheap and plain and probably somewhat inferior to the N.R.C. recommendations.

Program. For 10 weeks prior to the experiment proper the subjects were housed in the Laboratory where they were maintained on a fixed program of diet and activity so that the states of both nutrition and training were brought to substantially constant levels. During this time all the tests and measurements were standardized. A month before the start of the experiment proper the subjects were on a special regimen of high energy expenditure for 3 weeks

TABLE 1

Characteristics of the subjects at start of experiment

Age in years, height in inches, weight (nude) in pounds, and percentile score in the American Council on Education (A. C. E.) Psychological Examination, for College Freshmen, 1942 form

ITEM	GROUPS							
	Restricted				Supplemented			
Subject	G	Wi	Wa	T	S	Ja	N	Jo
Age	27	21	25	32	22	24	20	23
Weight	123	138	151	156	128	158	149	158
Height	68½	69½	71	71½	68¼	70½	67¼	74
A. C. E.	95	81	40	97	91	18	9	75

during which the intake of B vitamins was low for 2 weeks (cf. Keys, Henschel, Taylor, Mickelsen and Brozek, 1944).

In the experiment proper all men ate the same diet and daily ingested capsules of identical appearance. These capsules provided only vitamins A and D (daily 5000 I.U. vitamin A) for 4 men (the "restricted" group) but for the other 4 men (the "supplemented" group) they provided in addition, per day, 1 mgm. of thiamine, 1 mgm. of riboflavin and 10 mgm. of niacin-amide. The contents of the capsules were unknown to the subjects and to all but 3 members of the Staff who maintained secrecy.

The principal physical activity was provided by standardized daily tasks on the treadmill and outdoor walks over a fixed course. The remaining free time was covered by a schedule of maintenance and assistance work to which each man was assigned.

Nude body weight was recorded daily before breakfast. Once every 2 to 3 weeks all subjects went through a series of performance tests on the treadmill and a series of strength and psychomotor tests. At intervals of 1 to 2 months

the psychological and clinical examinations and measurements were made. All tests and measurements were made in fixed relation to time of day, meals, and previous activity. The test rooms were maintained at 78°F. and 50 per cent relative humidity throughout. Standard clothing was worn at all times.

Diet. All meals were prepared and served in the Laboratory by a dietitian and assistants. The great bulk of the food was supplied by the U. S. Army Quartermaster Corps in a single shipment of items selected from U. S. Expeditionary Force Menu no. 1. These materials were kept in a cool dry storehouse throughout the experiment. Frozen chickens and veal were kept at −10 to −15°C. Special 70 per cent butterfat cream was supplied from the University Farm; this was diluted 1 part to 3 parts of water for coffee and cereals and 1 part to 1 part of water for whipped cream desserts. Butter, celery, lettuce, radishes and small quantities of fresh fruits were bought on the local market. All breads and pastries were baked in the Laboratory, using special unenriched flour. Bread was fortified in protein and minerals with 10 per cent of vitamin-free casein, 0.72 per cent of tri-calcium phosphate and 0.01 per cent of ferric citrate.

Ten menus were strictly followed in rotation and all portions were weighed. Extra identical meals for each day were rapidly and carefully ground, sharp frozen and stored at −25°C. The meals from 3 to 5 days were combined for vitamin analysis.

The diet provided an average of 3300 calories and 75 grams of protein daily; fats comprised about 35 per cent of the calories. The foods for 3 representative menus are listed in table 2. The diet was designed to provide, per 1000 Cal., about 0.18 mgm. of thiamine, 0.25 mgm. of riboflavin, and 3.5 mgm. of niacin but some variations occurred. The analyses showed grand averages of 0.185, 0.287, and 3.71 mgm. of these vitamins respectively. The results of the actual vitamin analyses are summarized in table 3.

Methods. In general, all methods were the same as previously described (cf. op. cit.). All chemical analyses were made in duplicate and were controlled by analyses of known materials in collaboration with the National Research Council Committee on Food Composition.

Urinary excretion. Full details of the studies on urinary excretion of the vitamins will be reported elsewhere. The general results for the 24-hour excretions of thiamine and riboflavin, as percentages of total intakes, are summarized in table 4.

The thiamine excretion in both "restricted" and "supplemented" groups declined rapidly to reach substantially constant levels within 19 days, subsequent values showing no consistent trend. Zero excretions of thiamine, that is, where the "blank" value equalled or exceeded the unknown, occurred frequently in the "restricted" group beginning in the fourth week, and represented 36 per cent of all measurements in the last 20 weeks of the experiment. There were some consistent differences between individuals but these were not very large. In 14 sets of measurements on the "restricted" group in the last 20 weeks the 4 different individuals excreted averages of 7.7, 11.0, 8.3, and 10.0 micrograms of thiamine per day. In the 4 "supplemented" men at the same time, the different

TABLE 2

Representative diets

DIET NO. 2	DIET NO. 6	DIET NO. 10
100 grams pear sauce	100 cc. grapefruit juice	100 grams apricots (dried)
20 grams cornflakes (unenriched)	20 grams rice krispies	20 grams cornflakes
75 grams cream (35%)	75 grams cream (35%)	75 grams cream (35%)
80 grams roast chicken	125 grams beef broth	150 grams cr. codfish
20 grams dressing and gravy	150 grams corned beef hash	70 grams biscuit
150 grams mashed potatoes	150 grams hominy	150 grams jello salad
100 grams green beans	25 grams pickles	75 grams pineapple ice
65 grams angel food cake	100 grams jello with wh. cr.	30 grams cookies
75 grams pineapple ice	30 grams cookies	75 grams meat loaf
80 grams corned beef sandwich	130 grams croquettes (chicken)	150 grams fried potatoes
125 grams fruit	150 grams fried potatoes	100 grams creamed corn
55 grams cookies	100 grams spinach	100 grams spice cake
300 grams bread	125 grams gingerbread; wh. cr.	230 grams bread
50 grams butter	200 grams bread	50 grams butter
100 grams jelly	50 grams butter	100 grams jelly
	100 grams jelly	
Total wet wt.: 1462 grams	1678 grams	1476 grams
Thiamin content: 0.45 mgm.	0.46 mgm.	0.732 mgm.
Riboflavin content: 0.68 mgm	0.84 mgm.	0.88 mgm.
Niacin content: 8.8 mgm.	9.6 mgm.	11.5 mgm.

The bread was made with unenriched flour and with casein as described in the text; the cream was prepared by diluting 70 per cent butterfat cream with water; all breakfast cereals were unenriched; the potatoes and fruits were prepared from dehydrated products, the meat loaf and croquettes were made from a minimum amount of meat and a maximum amount of bread and casein. WH. = whipped; CR. = cream.

TABLE 3

Daily vitamin content of the basal diet, in milligrams per 1000 Cal., and total milligrams per day, as determined by direct analysis. Only the averages for successive periods of about 1 month each are given here

PERIOD	MGM. PER 1000 CAL.			TOTAL MGM.		
	Thiamine	Ribo.	Niacin	Thiamine	Ribo.	Niacin
in days						
1 to 29	0.17	0.22	3.7	0.56	0.72	12.2
30 to 55	0.18	0.23	3.3	0.58	0.76	10.8
56 to 86	0.19	0.33	3.7	0.63	1.10	12.1
87 to 116	0.21	0.35	4.0	0.70	1.15	13.1
117 to 146	0.18	0.30	3.7	0.58	0.99	12.3
147 to 161	0.19	0.29	4.0	0.62	0.91	13.3
1 to 161	0.185	0.287	3.71	0.612	0.948	12.24

individuals excreted averages of 122, 124, 183, and 104 micrograms per day. During the preliminary control period, when all men were on the same intake, the men in the eventual "restricted" group excreted respective averages of 197, 208, 154, and 149 micrograms per day while those in the "supplemented" group averaged 194, 183, 265 and 136, respectively.

The urinary excretion of riboflavin was much less responsive to differences in intake. A clear difference between "restricted" and "supplemented" groups was not apparent until the ninth week and thereafter there was no significant further change in either group. Again, the individual differences were not large. For the period from the ninth through the twenty-fourth weeks the "restricted" subjects excreted averages of 155, 113, 134 and 128 micrograms per day while the "supplemented" averaged 439, 505, 491 and 535 micrograms per day, respectively.

TABLE 4

Urinary excretion of thiamine and of riboflavin as percentage of total intake

"R" is the average for the experimental "restricted" group; "S" for the "supplemented" group. Each value in the table is the average of 8 to 16 measurements over a period of several weeks as indicated in the column headings. "Prelim." values were obtained during the preliminary control period 4 to 6 weeks prior to the experiment proper.

ITEM AND GROUP	PRELIM. PERIOD	PERIOD, IN DAYS					
		21–28	29–56	57–77	78–98	99–133	134–161
Thiamine, R	11.3	2.4	1.0	2.1	2.8	0.3	1.1
Thiamine, S	12.6	6.0	5.4	9.7	8.9	7.0	8.5
Riboflavin, R	22.9	19.4	19.2	12.4	12.6	12.2	12.1
Riboflavin, S	25.2	19.6	23.6	27.0	18.1	25.0	22.5

The urinary excretion of F_2 (a metabolite of niacin), as determined both by the method of Najjar et al. (1942) and of Huff and Perlzweig (1943) showed no consistent differences between the 2 groups of subjects.

Body weight, basal pulse rate and basal metabolism. The body weight remained remarkably constant in all the subjects throughout the experiment. There was a slight adjustment to the new conditions amounting to an average decline of 2.9 pounds during the 55 days of the preliminary control period. At the start of the experiment proper the "restricted" and "supplemented" groups averaged 141.8 and 148.3 pounds, respectively. Twenty days later the averages were 140.5 and 146.5 pounds, respectively, and the averages at the end of the experiment were almost identical, viz., 140.5 and 145.5 pounds.

The basal pulse rate likewise showed no significant changes in either group. Just prior to the experiment proper the basal pulse averaged 53.5 and 52.0 beats per minute in the "restricted" and "supplemented" groups, respectively, while the corresponding averages for the eighteenth through the twenty-third week were 52.6 and 53.0.

There was a very slight but statistically significant tendency for the basal metabolism to decline in both groups, but this may represent increasing habituation to the procedure in spite of a number of practice trials during the preliminary control period. In any case there was no difference between the 2 groups. The average oxygen consumption in the preliminary control period was 231.7 and 235.7 cc. per minute in the "restricted" and "supplemented" groups, respectively; at the end of the experiment the corresponding values were 220.2 and 226.0 cc. per minute.

Work performance and physical "fitness". Capacity for simple physical exertion was estimated chiefly from pulse rates during and at set intervals following standard tasks on the motor-driven treadmill. The tasks were: 1, walking for 60 minutes at 3.5 miles per hour and 10 per cent rate of climb ("endurance"); 2, running for 90 seconds at 9.0 miles per hour and 8.6 per cent rate of climb ("anerobic" work); and 3, the Harvard Fatigue Laboratory physical fitness test (Johnson, Brouha and Darling, 1942). None of these disclosed

TABLE 5

Scores in the Harvard Fatigue Laboratory Physical Fitness Test

Each value is the average of 3 or 4 independent tests on each man. Only values representing the period just before and again at the end of the experiment proper are given here; i.e., all results from tests between these times are omitted for the sake of brevity.

"RESTRICTED"			"SUPPLEMENTED"		
Subject	Before	End	Subject	Before	End
G	86	72	S	93	89
Wi	43	47	N	84	80
Wa	54	66	Jo	57	66
T	48	68	Ja	66	68
Mean.......	58	63	Mean.......	75	76

any significant differential effects of the diet. The pulse rate response to simple anerobic work was almost identical in the two groups. For example, at the end of the experiment the average pulse rates for the periods 0 to 15 seconds, 60 to 75 seconds and 120 to 135 seconds after "anerobic" work averaged, respectively, 184, 147, and 130 in the "restricted" group and 189, 149 and 127 in the "supplemented" group.

Differences in native ability were more pronounced in the Harvard "fitness" test but there was no important consistent change during the course of the experiment. The men in the "restricted" group started at a lower level and apparently improved rather more than the "supplemented" men. A brief summary is given in table 5.

Strength. Strength, as estimated by both hand grip and back lift dynamometers, was substantially constant in all individuals and unaffected by the vitamin intake. At the start of the experiment proper the grip strength averaged 55 kgm. in the "restricted" group and 58 kgm. in the "supplemented" group;

during the last 2 weeks of the experiment these averages were 61 and 60 kgm., respectively. The final averages for back lift were precisely the same as at the start, namely, 156 and 176 kgm. in the "restricted" and "supplemented" groups, respectively.

Blood lactate and pyruvate. The resting blood lactate level was substantially constant and completely independent of the vitamin intake as judged from 28 measurements in duplicate. The resting blood pyruvate, however, tended to rise in the "restricted" group and the change in this group was statistically significant. The statistical analysis of the data is summarized in table 6 which gives averages for each group, together with the standard deviations and *t* values. It should be noted that the principal change took place within 2 or 3 months and that there was no subsequent progression. The great change in *t* value during

TABLE 6

Mean values for resting blood pyruvate, as milligrams of pyruvic acid per 100 ml. of whole blood, together with analysis of the significance of the changes after 2 to 3 months and after 5 to 6 months on the diet as compared with the preliminary control period ("before")

The Fisher t-test for paired variates was used with 3 degrees of freedom. The values of *t* at various levels of significance are included

GROUPS	BEFORE		2 TO 3 MONTHS			5 TO 6 MONTHS		
	Mean	σ	Mean	σ	t	Mean	σ	t
Supplemented	0.89	±0.03	0.99	±0.15	0.87	0.93	±0.02	0.49
Restricted	1.01	±0.17	1.18	±0.18	2.06	1.15	±0.15	6.43

Levels of significance

LEVEL	50%	10%	5%	1%
t	0.765	2.35	3.18	5.84

the 5 to 6 month period was due entirely to decreases in the dispersion of the pyruvate values within the "restricted" group.

The blood lactate and pyruvate after standard anerobic work tended to rise during the course of the experiment in both groups of subjects but the rise was more prominent in the "restricted" group and the increase in pyruvate was perhaps disproportionate to that of lactate (cf. table 7). Exact analysis is complicated by the fact that the two groups were rather different at the outset, the "supplemented" group being more "fit" as indicated by a lower lactate maximum and a more rapid recovery. Under such conditions simple lactate/pyruvate ratios are misleading as we have abundant evidence that this ratio is normally dependent upon the total lactate level. It does seem, however, that the initial difference between the two groups was accentuated by the difference in vitamin intakes, particularly at 30 minutes of recovery, and that there was a real effect on the relation between lactate and pyruvate.

After the ingestion of 100 grams of glucose (with 200 cc. of water) there was

usually a small rise in blood pyruvate, particularly in the 90 minute sample. There were only trivial and inconsistent differences between the 2 groups of subjects in this respect. In the last 2 weeks of the experiment the average rise in pyruvate 90 minutes after glucose was 0.17 mgm. per 100 cc. of blood in the "restricted" group and 0.19 mgm. in the "supplemented" group. On some occasions there was a decrease after glucose. For example, at 60 minutes after glucose in the third month of the experiment the blood pyruvate *declined* on the average by 0.04 mgm. per 100 cc., in "restricted" group, while the "supplemented" group showed an average *increase* of 0.12 mgm. None of these apparent differences was statistically significant.

Glucose tolerance. Data on glucose tolerance do not lend themselves to treatment by averages and, in the present experiments, provided little of significance. Similar to our previous experience there was a rather high degree of intra- and inter-individual variation. Relatively flat curves were common in

TABLE 7

Work recovery blood lactate and pyruvate, as milligrams of the acids per 100 cc. of whole blood

All values are averages from 2 or 3 independent experiments with 4 subjects in each group. Two sets of averages are presented, one for bloods drawn at 12 minutes and one at 30 minutes after the end of standard anerobic work.

GROUP AND TIME	PERIOD AND ITEM					
	Before		60–90 days		145–161 days	
	Lact.	Pyruv.	Lact.	Pyruv.	Lact.	Pyruv.
Restricted, 12 min	72	3.9	86	4.9	89	4.7
Supplemented, 12 min	60	3.8	65	3.9	71	4.1
Restricted, 30 min	43	2.9	50	3.7	62	3.6
Supplemented, 30 min	31	2.7	29	2.6	36	2.9

both groups and the infrequency of marked hyperglycemia may be related to our failure to demonstrate important increases in blood pyruvate in glucose tolerance tests. In any case the blood sugar response to glucose ingestion failed to show any trace of an effect of the vitamin intake.

Psychomotor functions. The psychomotor functions tested included: 1, speed of simple repetitive motion (alternate tapping of 2 plates separated by 1.7 cm.); 2, speed of whole body motion (choice gross body reaction time); 3, co-ordination with no premium for speed (pattern tracing), and 4, speed and co-ordination (pipe and ball test). In tapping, the number of taps in both the first 10 seconds and the last 10 seconds of a 30-second test were recorded separately. In pattern tracing, both the number and the total duration of errors were recorded. The nature of these tests has been discussed previously (Brozek, 1944; Keys and others, op. cit.). All of these tests were applied during actual standard work and have proved reasonably sensitive to deleterious influences.

The average results from these tests are summarized in table 8. Statistical

analysis confirmed the absence of any effect resulting from difference in vitamin intake. The individual records of the several subjects likewise disclosed no differential trends. The normality of the practice effects was proved by numerous control studies on other individuals.

Sensory functions. Visual and kinesthetic sensory functions were covered to some extent in the several psychomotor tests. In addition there were measurements of the critical frequency for fusion of a flickering light, estimates of pressure and vibratory sense by the examining neurologists and the results of standard ophthalmological examinations. All of these tests and examinations were

TABLE 8

Average results in tests of psychomotor functions

Test 1—total number of taps in 10 seconds. Test 2—time in units of 1/120 second. Test 3A—total number of errors (contacts) in one circuit of pattern. Test 3B—total duration of errors (contacts) in one circuit, time in units of 1/120 second. Test 4—number of balls passed through the pipe in 60 seconds. Note that high scores indicate good performance in tests 1 and 4 but poor performance in tests 2 and 3.

TEST	GROUP	PRELIM. CONTROL PERIOD	PERIOD IN DAYS			
			45	78	117	141
1A. Tapping, 1st 10 secs.	"Restricted"	67	73	73	69	71
Tapping, 1st 10 secs.	"Supplemented"	64	70	75	71	72
1B. Tapping, 3rd 10 secs.	"Restricted"	62	63	64	63	63
Tapping, 3rd 10 secs.	"Supplemented"	58	60	64	61	61
2. Body Reaction Time.	"Restricted"	45	49	55	56	51
Body Reaction Time.	"Supplemented"	43	46	50	52	52
3A. Co-ordination, No. of errors.	"Restricted"	31	28	26	22	19
Co-ordination, No. of errors.	"Supplemented"	38	42	33	31	22
3B. Co-ordination, Duration	"Restricted"	162	130	101	90	79
Co-ordination, Duration.	"Supplemented"	199	210	146	133	94
4. Ball-Pipe Test.	"Restricted"	74	75	76	76	76
Ball-Pipe Test.	"Supplemented"	65	67	68	70	72

applied with care to all subjects on repeated occasions during the course of the experiment. None of them showed any consistent or significant alterations in either the "restricted" or the "supplemented" group during the course of the experiment.

Intellective functions. An attempt was made to assess possible effects of the level of vitamin intake on intellective functions by the use of 2 different "intelligence" tests and with the Porteus Maze, testing "foresight." These were applied twice in the preliminary control period (4 to 6 weeks and 2 to 3 weeks prior to the experiment proper), and on 3 occasions during the experiment proper. The American Council on Education Psychological Examination (A.C.E.) forms

for 1942, 1941, 1940, 1939 and 1938 were used in that order. The Ohio State University Psychological Test (O. S. U.) forms 21, 20, 19, 20, 19 were used in that order. The Porteus Maze in orientation 1 was used on each occasion as well as, on successive occasions, orientations 1, 3, 2, 4, 3 in that order.

The essential average results from these tests are summarized in table 9. Precise comparisons are difficult in this area because of differences in original native ability, variations in relative difficulty of successive tests, and some unavoidable learning effects. However, these sources of potential error actually gave less trouble than anticipated and the final results seem to have considerable

TABLE 9

Average results in tests of intellective functions (see text)

A. C. E. Psychological Examination raw scores based on 200 possible correct answers. O. S. U. raw scores. Porteus Maze point scores and time scores in seconds for orientation 1 (1), and for maze orientations 1, 3, 2, 4, 3 (1–4) in that order.

ITEM	GROUP	PERIOD				
		Prelim. control		Days of experiment		
		1	2	44–55	83–88	145–146
A. C. E. "Intelligence"	"Restricted"	128	153	148	145	136
A. C. E. "Intelligence"	"Supplemented"	101	117	120	115	114
O. S. U. Intelligence...	"Restricted"	118	126	120	128	122
O. S. U. Intelligence...	"Supplemented"	82	91	93	91	93
Porteus Maze (1), point score	"Restricted"	43	47	45	52	47
Porteus Maze (1), point score	"Supplemented"	43	51	50	52	49
Porteus Maze (1), time score.......	"Restricted"	276	192	232	154	164
Porteus Maze (1), time score.......	"Supplemented"	242	173	165	132	144
Porteus Maze (1–4), point score.....	"Restricted"	43	46	46	50	47
Porteus Maze (1–4), point score.. ..	"Supplemented"	43	48	46	46	50
Porteus Maze (1–4), time score.	"Restricted"	276	232	205	168	183
Porteus Maze (1–4), time score......	"Supplemented"	242	210	178	174	129

validity. We believe they demonstrate clearly that no important changes in intellective functions occurred in either group.

Personality. Close daily personal contacts with the subjects did not disclose any consistent or important alterations of personality or abnormal subjective complaints. More definite evidence was obtained by the use of self-rating questionnaires which covered the following items: Headache, flightiness, tiredness, out of patience with group members, sleeplessness, decline of mental alertness, decrease of interest in experiment and work, nervousness, sluggishness, backache, blueness, irritability with work, sensations of hot and cold, loss of appetite, "don't care" attitude, irritated by staff, forgetfulness, dizziness,

muscle soreness. Each of these items were checked as: "absent or normal," "more than usual," "quite a bit," or "very much so," and these were given respective numerical scores of 0, 1, 2 and 3. Results, as average sums of the item scores, are summarized in table 10. Both groups tended to have some increase in complaints but this was very slight and there was no appreciable difference between the 2 groups.

Inter-individual ratings were used to see whether the different subjects observed any changes in the behavior of their fellows. In the judgment of their fellow subjects every man in both groups was considered to have become less "normal" in the course of the experiment but none of these reported changes was large and there was no consistent significant difference between the 2 groups.

The Minnesota Multiphasic Personality Inventory (McKinley and Hathaway, 1943) was applied to all subjects twice in the preliminary control period and on 3 occasions during the experiment proper. Scores were computed for the following scales: hypochondriasis, depression, hysteria, psychopathic deviation, masculinity-femininity, paranoia, psychasthenia, schizophrenia and hypomania.

TABLE 10

Symptoms reported in the self-rating questionnaire

Average *sum* of entries, weighted as indicated in the text. The average of 2 questionnaires filled out within 4 weeks was used in each case, the days noted being the mid points of these occasions.

GROUP	PERIOD			
	Prelim. control	Days on diet		
		45	95	135
"Restricted"	1.50	2.88	2.88	2.25
"Supplemented"	1.25	3.10	1.75	1.75

Space does not permit even a cursory presentation of the extensive results but, in general, each subject remained within the score limits established for himself in the preliminary control period and there were no consistent alterations which might be related to the diet.

R. B. Cattell's Cursive Miniature Situation (C.M.S.) Personality Test (Cattell, 1941) was applied each month during the experiment proper but was not standardized in time to use in the preliminary control period. The results revealed a small steady increase in total correct responses in all but one man, a control subject, whose performance became slightly worse. There was no indication of deterioration in the individual "restricted" subjects and their group averages started at a higher level and improved somewhat more than the "supplemented" men.

The Rorschach ink-blot test was applied (Harrower-Erickson and Steiner, 1943) on the 25th and 151st day of the regimen. The records were scored and interpreted by Doctor Harrower-Erickson, who had not seen the subjects and did not learn of their nutritional status until after her analysis had been com-

pleted. There were no indications of personality deterioration in any subject. Three of the four restricted subjects, however, did give evidence of a slight increase in conscious control and vigilance over their behavior. This is not a psychopathological tendency, but it might be one way of reacting to less favorable dietary conditions.

Miscellaneous examinations. Clinical examinations were made on all men before and at intervals during the experiment proper. These included, besides the usual physical examination, particular attention to the skin, mouth and eyes. The latter were carefully examined with the slit lamp and ophthalmic microscope. No abnormalities conceivably related to the diet were discovered in any of the subjects at any time. Corneal vascularity, Bitot's spots, cheilosis and lingual changes were conspicuously absent.

Special cardiac examinations included measurement of heart size by means of special roentgenkymographic methods (cf. Keys, Friedell, Garland, Madrazo and Rigler, 1940). No significant changes occurred in any of the men. The same was true of the results of repeated electrocardiographic examinations. Carefully standardized measurements of gastric motility disclosed no changes in either rate of gastric emptying or the time for complete emptying of the stomach.

Discussion. The data actually presented in this paper are, because of space limitations, only a very small fraction of those collected in the course of this experiment. We believe they cover the several aspects of physiological, biochemical and psychological functions and "fitness" in a considerably more objective and exhaustive manner than has been reported previously and permit some conclusions of interest. However, it must be noted that the evidence applies strictly only to relatively normal young men under conditions of moderate activity in temperate climatic conditions. The situation may conceivably be different with women, children, the aged, and in the presence of disease. Extremes of climate, activity and emotional states may be modifying factors though most speculations on these matters have little or no basis of fact. A much more prolonged experimental period may have given different results though we may point to the absence of progressive tendencies after the first 10 weeks or so.

With the foregoing limitations in mind it seems clear that none of the performances or clinical or psychological characteristics was affected by the limitation in vitamins of the B complex to the rather low levels employed. There was, however, a small but statistically significant change in pyruvic acid metabolism. This took place within the time period employed in our previous studies on thiamine limitation and showed no progression thereafter. The obvious conclusion from this and our previous studies would be that the present level of thiamine intake, i.e., 0.185 mgm. per 1000 Cal., is not quite adequate to preserve full normality in the intermediary metabolism of carbohydrate but that an intake of 0.23 mgm. of thiamine per 1000 Cal. is fully adequate in this respect. There is no basis for judgment as to whether or not this small shift in pyruvate reaction equilibrium is, *per se*, deleterious, i.e., whether it is actually "bad," though it may be a warning signal.

It may be, of course, that the concomitant limitation in the other members of the B complex had a secondary effect on the pyruvate change through some influence on the requirement for thiamine. In any case it is clear that there was no indication that the intakes of riboflavin and niacin were inadequate. The findings in our previous work on riboflavin (Keys, Henschel, Mickelsen, Brozek and Crawford, 1944) are fully confirmed.

The number of subjects was smaller than we should like but confidence is gained by the uniformity of responses of the different individuals in spite of considerable differences in physique, temperament and previous habits of diet and activity.

The present results in the psychological field are of particular interest in view of the frequent suggestions and claims that intellectual ability, personality and behavior are peculiarly sensitive to the intake of B vitamins, notably thiamine (cf. Williams and others, 1942; Williams, Mason and Wilder, 1943). The present tests and results have some general importance in the methodology of applied psychology and will be presented with full details elsewhere. We are well aware of the fact that our observations and tests neither cover the entire field nor are as sensitive as desired, but we do believe they approach the best level of currently attainable procedure.

Exhaustive comparison of the present findings with the reports of other investigators is not warranted, partly because the conditions are not entirely comparable, but chiefly because of the relative paucity of precise objective data. The excretion of thiamine here remained well down in the range frequently considered to be indicative of gross deficiency. However, this was also true of the excretions reported in our previous study with an intake of 0.23 mgm. of thiamine per 1000 Cal. It should be noted that in the present series the thiamine excretion was consistently less than half that obtained with the 0.23 mgm. intake and frequently was undetectably small. It could be suggested, therefore, that a consistent thiamine excretion of less than 20 micrograms in 24 hours is related to true thiamine deficiency and that greater excretions indicate safety though not necessarily with a reasonable reserve margin.

The significance of riboflavin excretion data is not clear. From the present and previous studies here it might be suggested that a consistent excretion of around 100 micrograms per day would indicate adequacy. However, such a conclusion would not be on safe ground because of the delay in response to the intake of riboflavin excretion and because other factors undoubtedly are involved at times. For example, in other experiments in this Laboratory we have found that starvation, bed rest and total exclusion of thiamine from the diet all have very pronounced effects on riboflavin excretion. The present results are in good agreement with those of the Mayo Clinic studies on riboflavin though we are unwilling to accept their conclusions based on urinary excretion (Williams and others, 1943).

With thiamine the situation is more complicated. On the basis of experience we are forced to reject completely conclusions based solely on clinical impressions and uncontrolled observations in spite of the fact that these may be extremely valuable at times. The urinary excretion of thiamine is probably a significant

datum in the appraisal of thiamine nutrition but we cannot agree that the available evidence is sufficient to set a level of discrimination between true adequacy and inadequacy even though relative degrees of adequacy may be so assessed.

Some evidence has been offered which purport to show that a thiamine intake of 0.23 mgm. per 1000 Cal. is not fully adequate, or at least allows a shift in the equilibrium in pyruvic acid metabolism (Williams and others, 1942; Williams, Mason and Wilder, 1943). It is entirely possible that the whole source of apparent disagreement may reside in the calculation of thiamine intake in terms of milligrams per thousand Calories. Calculations may be made on the basis of non-fat calories, in which case the results from the Mayo Clinic and from this Laboratory would be less discordant because there was proportionately more fat in the present diet. Incidentally the diet used here was probably closer to the proportion of fat in the "average" diet in this country as a whole than was the Mayo diet. However, even a correction for this difference would still leave a considerable difference in the 2 sets of results. We question whether the thiamine requirement is actually strictly and linearly proportional to the calories or even to the non-fat calories. The evidence for this common assumption is not very satisfactory even in animal experiments and there is extremely little basis for presuming such an exact relation in man. If we merely examine all the available evidence without prejudice then we should probably conclude that signs of thiamine deficiency are apt to appear within some months when the total intake is less than 0.61 mgm. per day, regardless of calories. It is possible that the actual thiamine requirement may be a constant plus an amount proportional to the total or non-fat calories. This problem requires much more intensive study.

SUMMARY

1. Four normal young men were maintained on a controlled dietary intake of 0.185 mgm. of thiamine, 0.287 mgm. of riboflavin and 3.71 mgm. of niacin per 1000 Calories. Four other subjects were on the same diet but supplemented each day with 1 mgm. of thiamine, 1 mgm. of riboflavin and 10 mgm. of niacin. The difference in vitamin intake was disguised by the use of placebos. The actual intakes were determined by direct analyses.

2. All conditions and measurements were highly standardized and controlled. After several months of preliminary standardization the experiment proper was maintained for 161 days. The men resided in the Laboratory throughout the entire period. The physical expenditure of the men was adjusted so that a daily intake of 3300 Calories just maintained their weights.

3. The 24-hour urinary excretion of thiamine became substantially constant in less than a month in both restricted and supplemented groups. For the last 3 weeks of the experiment it averaged 7 micrograms (1.1 per cent of the intake) and 114 micrograms (8.5 per cent of the intake) in the 2 groups, respectively.

4. The 24-hour urinary excretion of riboflavin became substantially constant in about 2 months in the restricted group. In the supplemented group there was no tendency toward any progressive alteration from the beginning. For the last 3 weeks of the experiment the daily excretion of riboflavin in the urine

averaged 137 micrograms (12.1 per cent of the intake) and 438 micrograms (22.5 per cent of the intake) in the 2 groups, respectively.

5. Over the period of 161 days the 2 groups disclosed no differential changes in the following: pulse rate during and following both endurance and brief exhausting work, Harvard "physical fitness" test scores, strength, psychomotor functions, heart size, electrocardiograms, gastric emptying, basal metabolism, glucose tolerance, blood lactate in rest and following standard work or glucose ingestion, sensory and intellective functions, and a variety of objective personality evaluations. All clinical, ophthalmic and neuropsychiatric examinations were also negative.

6. The resting level of pyruvic acid in the subjects on the low vitamin intake increased from an average of 1.01 mgm. per 100 ml. of blood at the start of the experiment to 1.15 mgm. at the end and this change was statistically significant. There was a slight tendency toward a similar difference in the 2 groups after brief exhausting exercise. No differential change in blood pyruvate occurred following the ingestion of glucose.

7. Conclusions from the present study must be confined to the kind of subjects and the general conditions used and to the duration of the experiment. Within these limitations it appears that the vitamin restriction was without significant effect on the aspects of "fitness", "health" and personality studied here but that the thiamine intake was on the borderline of inadequacy as indicated by blood pyruvate. There were no signs of deleterious effects from the restriction in riboflavin and niacin.

Part II. The results of acute deprivation of vitamins of the B complex

The results reported in part I of this paper might suggest that the lowest intake of B vitamins studied—roughly one-third of the National Research Council Recommended Daily Allowances (1943)—fail only in trivial respects to cover all requirements for normal young men. The fact that the few and apparently unimportant changes which were observed were not progressive after the first 2 or 3 months affords no support for the speculation that subsistence on the diet for a period longer than 6 months would eventually result in deterioration.

Analogy with the reports of Frazer (1942) and Elvehjem (1944) that signs of nutritional deficiency (in the monkey) may be delayed for many months is not valid because in the cases cited no effort remotely comparable to the present study was made to detect malfunction at an early date. However, there are several important questions:

1. Are the tests and measurements used here properly sensitive to the functional deterioration produced by real deficiency?
2. What are the quantitative characteristics of such deficiency in terms of these and similar objective tests and measurements?
3. Do the restricted intakes of B vitamins employed result in an important decrease in body reserve of these vitamins so that subsequent more acute restriction produces more rapid or more serious deterioration than would obtain otherwise?

4. Which of the several B vitamins is most critical in the production of deterioration in short periods of extremely severe restriction?

An attempt was made to answer these questions in part by an experiment with a diet almost devoid of B vitamins. Immediately upon the completion of the 161-day experiment reported in part I of this paper these same subjects began subsistence on a diet which was adequate in calories, protein, fat, minerals and vitamins other than those of the B complex. The level of energy expenditure was set at 4000 Cal. daily and the diet was maintained for 33 days. Suitable controls were established and great efforts were made to discover in quantitative terms the main features of responses covering a considerable sector of the physiological, biochemical and psychological functions. A summary of the more significant findings is set forth here.

The program. In order to obtain evidence as to the effect of the previous diet on the response to a diet substantially free from vitamins of the B complex, and to provide both positive and negative controls, the 8 men were divided into 2 groups receiving, respectively, placebos and supplements of B vitamins as

TABLE 1

Organization of subjects according to the B vitamin intake for the 6 months previous to the present experiment and for the present experiment (33 days)

"Supplemented" means that intake of the vitamins of the B complex corresponded roughly to National Research Council Recommendations for the entire period indicated: "deficient" refers to the basal diet plus placebos only.

SUBJECTS	GROUP DESIGNATION	PREVIOUS	PRESENT
G, Wi	Restr.—Deficient	Restricted	Deficient
S, Ja	Control—Deficient	Supplem.	Deficient
J, Jo	Control—Control	Supplem.	Supplem.
Wa, T	Restr.—Control	Restricted	Supplem.

indicated in Table 1 of this terminal experiment. The increased frequency and variety of the daily tests, combined with the fixed schedule of exercise on the treadmills, made a routine with almost no free time and fulfilled the plan of an energy expenditure of 4000 Calories per day. All men ingested capsules daily and none of the subjects and only a few administrative members of the Staff knew which men were controls. As before all subjects resided continuously in the Laboratory. Every effort was made to assure both technical and biological accuracy of the tests and measurements. As in the previous work, clothing, temperature, humidity and other environmental conditions were kept constant.

The efforts to maintain absolute constancy of food intake and activity eventually were unavailing in the face of mounting anorexia and deterioration of some of the men after several weeks so that it became necessary to scale down both the food prescription and the exercise schedule. The schedule of all tests and measurements was not interrupted, however, except in the case of one man (Ja) who was withdrawn from the experiment after 18 days because of an upper respiratory infection.

On the 23rd day all men received vitamin test doses and on the following day thiamine supplementation was begun with the men not previously supplemented. This administration was decided upon because of accelerating debility and nausea of these men. The basal diet was adhered to until the end, however, and no other vitamin supplementation was introduced.

Methods. The chemical, physiological and psychological methods were, for the most part, those applied in part I of this paper. A number of tests and measurements not used previously were applied in the present experiment. These are indicated in the appropriate sections below. It should be noted that all chemical analyses were made in duplicate and checked against known standards. The tests and measurements of strength and in the sensory and psychomotor areas were applied in duplicate or in triplicate on each occasion.

Diet and nutrient intakes. Previous experimentation with the synthetic diet of "Crisco," "dextrimaltose" and casein used by Najjar and Holt (1943) indicated

TABLE 2

General formula for the diet

Weights in grams as supplied except for items 5, 6, 7 and 8 which are for the dry ingredients

ITEM	BKFST.	LUNCH	DINNER	TOTAL	CHO	PROTEIN	FAT
1. Butter	23	30	22	75			61
2. Syrup		100		100	70		
3. Jelly	75		75	150	97		
4. "Cream" (17.5%)	30			30			
5. "Wawfuls"		100		100	65	12	18.5
6. "Regurgicake"	150		150	300	195	36	60
7. Frozen pudding			100	100	65	12	18.5
8. Cookies			50	50	33	6	9
9. Soda Pop		175		175	25		
Grand total = 4014 Cal.							
Total grams					550	66	172.2
Total calories					2200	264	1550

we could not hope to maintain an intake of 4000 Cal. daily with this ration in subjects who have at least a moderate amount of gastronomic sensitivity. The diet eventually developed bore a reasonable resemblance to more customary foods and was neither seriously disagreeable in taste nor unbearably monotonous. It must be admitted that after some weeks it failed to arouse any enthusiasm.

The food items were butter, hydrogenated vegetable shortening ("Spry"), vitamin-free casein, dextrose, sucrose, cornstarch, sugar syrup, hard candy, jelly (apple-raspberry and apple-currant), and special "cream" prepared from 70 per cent butterfat. To these were added very small flavoring amounts of cinnamon, cocoa, lemon juice and fat bacon as well as coffee, black tea and coca cola as beverages. The general formula of the diet is given in table 2 from which it will be seen that it provided about 4000 Calories, 66 grams of protein, and fat amounting to 38 per cent of the calories.

Variation was introduced by the use of different flavorings in the several prepared items and by the substitution of beverages made from the puddings. The recipes and vitamin contents of specially prepared items are given in table 3. In general the diet covered both the desire for calories and the actual needs but hard candy was provided as an optional supplement if desired; small amounts were eaten (and recorded) by some of the men.

TABLE 3

Recipes for principal items used in Gook Diet no. 1

Note that spice or chocolate cookies may be prepared using 3 grams ground cinnamon or 5 grams cocoa instead of caramelizing sugar. Recipe amounts in grams, vitamins in micrograms per one recipe.

	WAWFUL	REGURGICAKE	CHOC. PUDDING	CARAMEL PUDDING
Cornstarch	44	50	9	40
Dextrose	21			
Sucrose		15	51	28
Casein	12	12	12	12
Butter	10		10	10
Shortening	10	20	10	10
NaCl	3	3		3
Baking powder	tsp.	tsp.		tsp.
Water	100	50	50	50
Calories	400	488	455	487
Thiamine	0.8	1.0	4.5	0.8
Riboflavin	0.5	0.5	15	0.5
Niacin	0.0	1.0	120	1.0

Notes: *"Wawful":* Mix dry as for pie crust, add water just before baking in usual manner· Makes 2.

"Regurgicake": Mix dry as for biscuits, add water and knead 1 minute. Bake at 425°F. for 30 minutes. Best served hot, fresh or toasted.

Chocolate pudding: Mix dry ingredients plus 3 grams salts no. 4 (Phillips and Hart, 1935) and 5 grams cocoa. Add boiling water, mix until smooth. Cook in double boiler 30 minutes, cool and freeze in mold.

Caramel cookies: Caramelize sugar, add butter and then water and cook until smooth. Cool and add mixed dry ingredients. Form into roll and slice off cookies. Bake at 400°F. for 20 minutes.

Chocolate-Cola drink: Place in Waring Blender 100 grams of chocolate pudding, 4 crushed ice cubes, ½ bottle (3 oz.) of coca-cola, 3 tablespoons liquid coffee. Mix 1 minute and serve.

All foods were repeatedly analyzed as served. The precise values for the B vitamins are somewhat uncertain, owing to the difficulty of estimation, even with special methods, at the very low levels present. Assurance can be had, however, that the total diet did not exceed the following values: Thiamine—0.03 mgm. per day, or 0.008 mgm. per 1000 Cal., riboflavin—0.05 mgm. per day, or 0.013 mgm. per 1000 Cal., niacin—0.4 mgm. per day, or 0.1 mgm. per 1000 Cal.

Each day all men ingested a series of capsules which provided, daily, 25 mgm. of ascorbic acid, 5000 I.U. of vitamin A and 170 I.U. of vitamin D for each man. In the deficient group the remaining capsules contained only lactose and coloring matter but the men in the control group received capsules of identical appearance containing 1 mgm. of thiamine, 1 mgm. of riboflavin, 10 mgm. of niacin, 25 mgm. of pyridoxine, and 1.2 grams of dried yeast so that their total intakes were, daily, 1.5 mgm. of thiamine, 1.5 mgm. of riboflavin and 10.8 mgm. of niacin. Mineral adequacy was provided to all men by the use of the salts mixture no. 4 of Phillips and Hart (1935).

The diet caused constipation in all men at one time or another. This was controlled by restricted use of mineral oil. As the experiment progressed anorexia became a problem and deviations from the exact form of the diet were allowed in an effort to cater to individual preferences. Such deviations, however, were confined to the substitution of puddings for "wawfuls" or "wawfuls" for "regurgicakes" and so on, so that the dietary pattern remained essentially constant. The actual total amount of the diet eaten, however, declined somewhat for all men after the first 10 to 15 days and fell to very low values in the third week in the deficient group. After thiamine supplementation of the latter group their food intake rapidly rose so that for the final week of the experiment all of these men averaged more than the prescribed 4000 Cal. per day; this was provided by eating extra servings of the several items of the diet.

General results. Aside from minor complaints about the monotony of the regimen no subjective or objective changes other than in urinary excretion of vitamins were discovered in any of the men in the first week. Thereafter there was increasing anorexia in G and Wi and later in S and Ja. This progressed in G and Wi to almost complete inability to take any food by the end of the third week. It was decided, therefore, to carry out vitamin saturation tests and then to institute supplementation. Accordingly, at the end of the 23rd day all men in the deficient group were given saturation test doses, orally, of 1 mgm. of thiamine, 1 mgm. of riboflavin and 10 mgm. of niacin. On the following day, the 24th on the diet, these men began to receive thiamine supplementation at the level of 10 mgm. per day for 5 days and 5 mgm. per day for the final 5 days. No other vitamin supplementation was given because the thiamine alone appeared to be sufficiently effective in restoring both appetite and general ability to proceed with the experiment. The body weight records summarized in figure 1 reflect the variations in the food intake quite accurately.

The changes in performance were surprisingly small in all categories in view of the marked changes in general behavior and obvious signs of subjective distress. In general, however, the objective findings were consistent and permit fairly definite conclusions. The stability of the supplemented group provided satisfactory controls.

The detailed clinical examinations of the deficient group were largely negative at all stages. In particular there were no changes in the skin, gums, mouth, tongue, eye grounds, cornea and conjunctiva. The objective neurological ex-

aminations were made with great care but they revealed only small evidence of peripheral nerve disturbance. The latter effect was limited to the lower legs and consisted in slight surface anesthesia and minor deep muscle tenderness in the calves. Subjectively there were reported occasional sensations in the legs of numbness, cold, weakness and pain on exertion. At the height of the effects the deficient men were uniformly apathetic, disinclined to either physical or mental effort and depressed. All time free from tests and other set features in the program was devoted to lying silently in bed. Vomiting during or between attempts to eat was frequent in these men and was often induced by exertion. It is notable that their lack of desire to eat was not limited to the experimental diet; for example, they were only mildly intrigued by the thought of beefsteak or fresh vegetables which they had not eaten for 7 months. It is interesting that the members of the Staff of the Laboratory who had been observing the

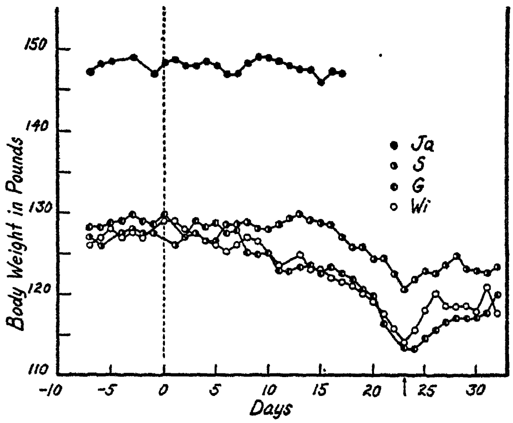

Fig. 1. Body weights nude, after emptying the bladder before breakfast, for the 4 men who received only placebos until the 23rd day when thiamine supplementation was started (arrow). Note that the 4 control subjects maintained relatively constant weights during this period.

men closely for many months were much more impressed with their deterioration than were any of the 5 outside examiners who conducted special clinical examinations.

Urinary excretion of the vitamins. The course of thiamine excretion in the urine, as averages for the 4 groups, is summarized in figure 2. The men, previously restricted, who received supplements (T and Wa) showed a general rise in thiamine excretion which was immediate, pronounced and constantly progressive. Since the total thiamine intake in these supplemented men was only 1.5 mgm., it is tempting to conclude that the body stores of thiamine in these men were depleted but easily replenished and that the supplement intake was considerably greater than needed. However, we disclaim any such definite conclusion and use this instance as an opportunity to reiterate our opinion that at present the interpretation of urinary excretion data for vitamins is frequently carried far beyond established legitimacy.

Thiamine excretion in the deficient group fell within a day to levels where it is necessary to work at the extreme limit of sensitivity for any methods so far developed. It can be stated, however, that G and Wi, the men previously on a restricted regimen, more closely approached absolutely zero excretion within a few days than did the men who were previously controls, i.e., S and Ja. The differences seem numerically trifling but they may reflect significant metabolic differences.

The course of riboflavin excretion was very different from that of thiamine both as to rate of change in response to change in intake and as to ultimate levels reached. The data are summarized in figure 3. The men previously restricted (Wa and T) showed no real response to supplementation for about 2 weeks. All men on the placebo regimen showed an immediate fall in riboflavin output but this was not progressive and appeared to become stabilized at a level of urinary output considerably in excess of the intakes. These latter

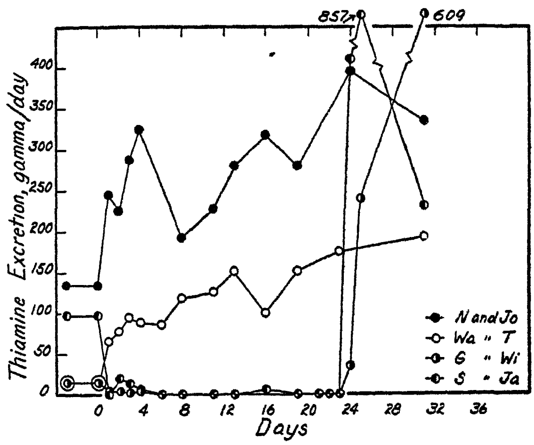

Fig. 2. Urinary excretion of thiamine, in micrograms per 24 hours, as averages for the 4 pairs of subjects.

results suggest either that the body stores of riboflavin are very great or that synthesis of riboflavin occurs in the body (Najjar and others, 1944). We should probably incline to the latter suggestion in view of the fact that, after the first 2 or 3 days, there was no essential difference in urinary riboflavin excretion between the men who had been, for nearly 6 months previously, on very different levels of intake.

Measurements of F_2 in the urine, as a proposed measure of niacin metabolism, were made on all the urines collected. They showed no important relation to intake and the results conform to our report as to the lack of utility of this measurement in men on low or moderate intakes of niacin (Mickelsen and Erickson, 1944).

The responses in the 24-hour urinary excretion to the "saturation" test doses of the vitamins administered to the deficient group on the evening of the 23rd. day were likewise different for the several vitamins. None of the ingested thia-

mine was excreted whereas an average of 10 per cent of the test dose of riboflavin appeared in the urine. The test dose of 10 mgm. of niacin produced no consistent change in the F_2 excretion though there was an average rise in the F_2 from 559 to 736 units (equivalent to micrograms of quinine sulfate); in other words an increase in intake of 2500 per cent produced an average rise of only 32 per cent.

Urinary creatinine and urea. During the present experiment there was a consistent tendency, in both groups, for the total 24-hour urinary creatinine to be slightly depressed as compared with the measurements made in the immediately preceding period. In any case it was clear that the vitamin deficiency, with the associated loss of weight and weakness, was not associated with any outpouring of creatinine. The average results are summarized in figure 4.

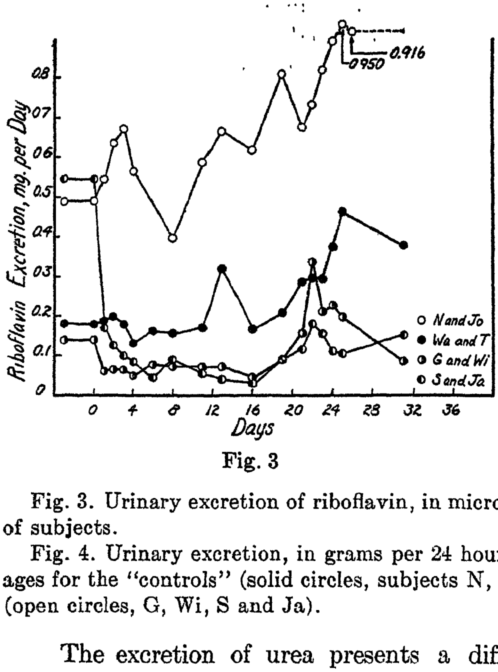

Fig. 3

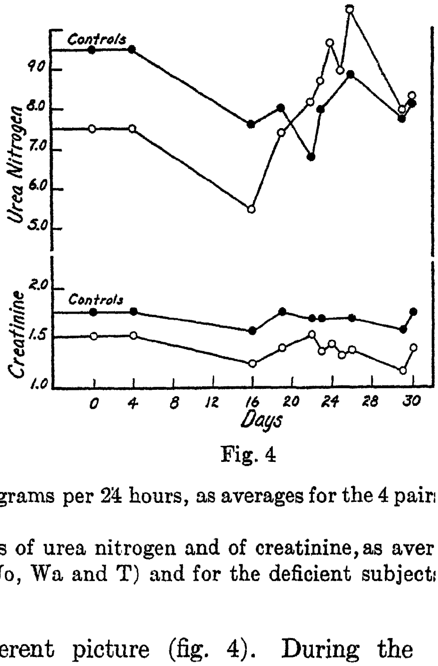

Fig. 4

Fig. 3. Urinary excretion of riboflavin, in micrograms per 24 hours, as averages for the 4 pairs of subjects.

Fig. 4. Urinary excretion, in grams per 24 hours of urea nitrogen and of creatinine, as averages for the "controls" (solid circles, subjects N, Jo, Wa and T) and for the deficient subjects (open circles, G, Wi, S and Ja).

The excretion of urea presents a different picture (fig. 4). During the first 2 weeks, when the signs of deficiency and metabolic alterations were slight, both groups behaved alike in exhibiting a reduction in urea output of about 25 per cent. At least part of this could be explained on the basis of the reduction in protein intake from an average of 75 grams to around 60 grams daily.[2]

After the first 2 weeks the control subjects maintained a fairly constant urea output at this new level. The deficient men, however, excreted increasingly large amounts of urea as their deficiency state became more severe. After thiamine supplementation this difference between the 2 groups tended to disappear. The results strongly suggest an important amount of tissue breakdown associated with the severest stage of the deficiency but final conclusions must be tempered by the absence of full data on the nitrogen balance.

[2] Note that the protein intake tended to fall somewhat below the prescription because of distaste for the casein and consequent rejection of part of the casein-containing items.

Blood chemistry. The deficient subjects showed a general tendency toward hemoconcentration more or less parallel to the course of their signs of deficiency. The plasma protein concentration was increased in G, Wi, and S, respectively, by 0.6, 1.1 and 1.5 grams per 100 ml. by the 22nd day with essentially no changes in the control subjects. The albumin-globulin ratios showed no important or consistent changes in either group. Evidence of the hemoconcentration changes is clearly seen in the values for hemoglobin summarized in table 4. Whereas the control subjects suffered small reductions in hemoglobin concentration in the middle of the experiment, all of the placebo subjects showed a progressive rise which regressed after they received the thiamine supplementation. It is probable these blood concentration changes simply reflected alterations in the state of hydration consequent to vomiting and partial starvation.

The major effort in blood chemistry was devoted to the study of lactic acid, pyruvic acid and glucose as possible indices of the behavior of the carbohydrate metabolism. These substances were repeatedly measured in rest, after standard "anerobic" work, and after glucose ingestion. The major results are summarized in table 5. The outstanding finding was the relative constancy of these variables

TABLE 4

Average values for concentration of hemoglobin, in grams per 100 ml. of blood, during subsistence on the critical diet

GROUP	CONTROL PERIOD	PERIOD IN DAYS		
		13	22	31
Supplemented subjects	15.25	14.58	14.40	15.23
Deficient	14.90	15.13	16.30	15.60

in spite of the abundance of signs of functional impairment. It is clear that, as compared with the pre-experiment control period or with the supplemented subjects, the deficient group exhibited a general and fairly consistent tendency to accumulate pyruvate and lactate, but in each instance the effect was small and, taken by itself, would not be considered important or even abnormal. In other words, though the data thoroughly establish an effect of deficiency of the B vitamins, and especially thiamine, on the metabolism involving lactic and pyruvic acids, the effect was too small to support claims for the validity of the diagnosis of athiaminosis from only a few measurements of these acids.

The sugar tolerance curves exhibited some interesting features which are rather obscured by the necessary condensation of the data into averages. At the height of the signs of deficiency all men in the placebo group showed a definite delay in disposing of the ingested sugar. The experimental regimen, however, also had some effect on the control subjects who showed definitely higher 30-minute levels than before. The reason for this general change is unknown. Some of the alterations in the deficient group were probably related to partial starvation.

Gastric emptying. We have remarked that anorexia, nausea and vomiting were among the earliest and most pronounced effects of the vitamin deficiency. It is interesting, therefore, that there was no delay in emptying the stomach as

observed with a standard meal of oatmeal and barium sulfate measured both by fluoroscopy and by planigraphic measurements of the serial roentgenograms (Henschel, Taylor and Keys, 1944). The final times of emptying of the stomach are given in table 6.

TABLE 5

Averages and standard deviations for lactate, pyruvate and glucose in milligrams per 100 ml. of blood

"Deficient" group-subjects G, Wi, S; "Supplemented" group-subjects N, Jo, Wa, T. All measurements are the means from the duplicate analyses. Measurements on 2 to 4 separate occasions for each man under each condition in each period except for the final period when only single measurements were made for glucose and for lactate and pyruvate after glucose. "Basal" refers to basal rest; "Standard" refers to standard rest after standard light activity. "12 min." and "30 min." refer to bloods drawn at 12 and at 30 minutes after 90 seconds at 9.0 m.p.h. and 8.6% grade on the treadmill. "30 min.", "60 min." and "90 min." refer to minutes after ingestion of 100 grams of glucose in 200 ml. Lactate and pyruvate are the increases observed at 90 minutes after glucose.

VARIABLES	PRE-EXPT. CONTROL				PERIOD							
					18–22 days				28–32 days (end)			
	Placebo		Control		Placebo		Control		Placebo		Control	
	Mean	±	Mean	±	Mean	±	Mean	±	Mean	±	Mean	±
Basal lactate...	7.98	2.88	6.09	1.08	6.83	1.97	6.70	1.41	11.1	3.07	7.23	2.42
Standard lactate.......					12.1	4.51	8.92	2.58	11.1	3.18		
Basal pyruvate.....	0.96	0.15	1.01	0.14	1.02	0.21	0.92	0.20	1.01	0.23	1.03	0.37
Standard pyruvate... .					1.59	0.45	1.27	0.21	1.48	0.58		
12 Min. lactate.	78.7	10.7	80.5	17.5	88.2	17.2	92.7	17.2	81.9	9.5	85.2	8.9
30 Min. lactate.	45.8	11.8	47.1	18.8	49.7	14.8	51.3	15.8	35.9	7.9	41.0	9.4
12 Min. pyruvate.....	4.27	0.67	4.39	0.66	5.09	0.50	4.72	0.57	4.27	0.46	4.90	0.13
30 Min. pyruvate.....	3.28	0.79	3.42	0.92	4.02	0.68	3.49	0.63	2.90	0.14	3.13	0.37
Lactate after glucose	+1.36	3.55	+4.46	2.05	+3.78	2.11	+2.27	1.79	−1.49	3.67	+3.13	1.24
Pyruvate after glucose......	+0.18	0.18	+0.31	0.24	+0.39	0.13	+0.09	0.15	−0.11	0.29	+0.24	0.11
Basal glucose..	70.8	5.7	72.7	3.5	72.2	6.8	72.7	3.0	64.7	6.7	77.0	1.9
30 min. glucose	90.0	11.5	103.5	14.0	101.0	11.2	122.7	14.3	73.2	6.0	115.2	13.9
60 min. glucose	88.1	11.4	87.3	25.7	101.8	27.3	88.2	16.5	95.6	9.7	104.4	25.8
90 min. glucose	70.5	12.9	72.0	13.1	92.1	25.6	73.8	14.3	83.4	4.0	69.6	5.8

Liver function. Attempts were made to discover possible indications of impaired liver function by means of a series of tests and measurements in the pre-experiment control period and at the time when deficiency signs were most manifest. No changes were found in the intravenous hippuric acid test (Quick, 1936, 1939), blood bilirubin (Malloy and Evelyn, 1937), phosphatase (Flood,

Gutman and Gutman, 1937; Weise and others, 1939) or prothrombin time (cf. Warner, Brinkhous and Smith, 1936; Quick, 1938). The cephalin-cholesterol flocculation (Hanger, 1939) was increased slightly in all of the deficient subjects but in only one of the 4 controls. This small indication of abnormality was offset by the negative results in clearance tests for phenolsulfonphthalein and bromsulfalein. It is obvious that any impairment in live function which may have been produced must have been very slight.

TABLE 6

Final emptying time, in minutes, of the stomach after a standard meal of oatmeal and barium sulphate

Measurements made before the critical diet, on the 14th day of the experiment, and after vitamin supplementation following the experiment

GROUP AND SUBJECT	BEFORE	14 DAYS	AFTER	GROUP AND SUBJECT	BEFORE	14 DAYS	AFTER
Defic. G	120	90	150	Control, N	90	90	90
Defic. Wi	90	120	60	Control, Jo	150	120	150
Defic. S	60	90	60	Control, Wa	150	210	120
Defic. Ja	90	105		Control, T	90	120	90
Defic., aver	90	101	90	Control, aver.	120	135	105

TABLE 7

Values for pulse rate, blood pressure and oxygen consumption in basal rest before and during (18th day) the experiment

Pulse in beats per minute, blood pressure in millimeters of mercury, B.M.R. in milliliters of oxygen per minute

SUBJ.	PULSE		SYSTOLIC BP		DIASTOLIC BP		B.M.R.	
	Before	During	Before	During	Before	During	Before	During
G	60	48	104	100	72	68	224	199
Wi	58	62	108	112	60	60	212	198
S	48	48	108	106	64	58	218	204
Ja	56	52	116	114	84	72	222	240
N	47	45	116	112	72	66	218	217
Jo	60	60	110	104	64	70	246	256
Wa	45	40	118	118	76	80	223	225
T	46	40	112	108	74	76	222	230

Cardiovascular and respiratory functions. Evidence as to cardiovascular and respiratory functions in work is discussed below in the section on physical "fitness". In rest these functions were little altered as indicated by pulse rates and blood pressures given in table 7. Subject G developed a resting bradycardia which is not very marked in view of the low pulse rates shown by most of these subjects in rest even on adequate diets. Low heart rates are characteristically shown by our well-trained subjects under our customary conditions of very complete relaxation in basal rest.

Electrocardiographic records revealed no significant alterations other than a

general tendency toward low voltage in the deficient subjects at the time of their most pronounced debility. The measurements of heart size are given in table 8. They show no trace of cardiac enlargement but rather, in subjects G and Wi, a pronounced diminution in the size of the heart at the time when their symptoms were most severe; there was apparently some return toward normal after 10 days of thiamine. This change may be related to the partial starvation induced by anorexia and nausea; we have generally observed a sharp reduction in heart size in starvation experiments.

The maximal capacity to transport oxygen and the amount of oxygen removed per liter of ventilation in maximal work are useful measures of total cardiovascular and respiratory function. Such measurements were made on the treadmill at 7.5 miles per hour and 8.6 per cent grade. Unfortunately the progressive illness of the deficient subjects, and particularly their nausea in extreme exertion, precluded the use of these measurements at the height of their symptoms but on the thirteenth day only subject G showed significant changes. These consisted in a decline in maximal oxygen transport to 3.00 liters per minute from a previous level of 3.28 and a fall in the milliliters of oxygen removed per liter of ventilation to 25.7 from the pre-experiment value of 38.5.

TABLE 8

Heart size, in milliliters of total volume, as measured by method of Keys and others (1940)

Column headings give the number of days on the diet

SUBJECTS	BEFORE	DAY 11	DAY 23	DAY 31
G	391	422	329	384
Wi	407	394	334	368
S	503	512	512	513
Ja	579	536		
N	440	426	459	448
Jo	501	450	482	465
Wa	643	636	674	655
T		546	578	539

Physical "fitness", strength and endurance. The difficulties in appraising physical "fitness" have been discussed elsewhere (Keys, 1943, 1944; Taylor and Brozek, 1944). In general, such appraisal involves one or more of the following: 1, strength; 2, apparent capacity to continue brief severe work; 3, apparent capacity to continue prolonged work; 4, pulse rate following brief severe work; 5, pulse rate during moderate work. Repeated observations in all these categories were made in the present experiment.

It has consistently been the experience of this laboratory that simple strength, as measured by such tests as grip strength and back or leg lift dynamometers, is extremely resistant to deterioration in relatively short periods of debilitating conditions. The present results offer no exception. Neither grip strength nor back lift suffered any significant change in any of the men with the exception of an apparent slight fall in grip strength in subjects G, Wi and S in the latter part of the experiment (table 10).

The pulse rate in standard aerobic work (3.5 m.p.h. and 10 per cent grade) was measured every 2 to 4 days, each measurement representing the average of 4 determinations in the last 10 minutes of a 60-minute work period. Only one subject showed any significant change before the completion of the work itself

TABLE 9

Scores in the Harvard Fatigue Laboratory Fitness Test

SUBJECT	PRE EXPT.	DAYS ON DIET							
		3	7	11	15	19	23	29	33
G	72	60	49	47	53	58	*	*	77
Wi	51	46	44	34	26	26	*	*	28
S	87	92	85	88	95	90	85	80	84
Ja	68	64	64	69	70	66			
N	79	81	78	81	80	82	74	74	79
Jo	71	58	67	69	81	85	76	62	50
Wa	48	52	63	59	61	64	59	60	59
T	38	50	44	48	50	50	46	55	55

* Impossible to carry out tests.

TABLE 10

Average values for the tests of strength, speed and co-ordination

Note that deterioration is indicated by decreasing scores in items 1, 2, 3, 4 and 8, and by increasing scores in items 5, 6 and 7. "Tapping Speed, Initial", and "Final" list the average number of taps per second in the first 10 and in the final 10 seconds of a 30 second test. "Co-ordination I" gives the number of errors and "Co-ordination II" the total duration of those errors in the pattern tracing test. "Speed and Co-ordination" gives the number of successful ball-pipe passages in the ball and pipe test. Group C is the control (fully supplemented) group of subjects N, Jo, Wa and T. Group D is the deficient group of the subjects G, Wi and S who received only placebos until the 23rd day and thereafter received thiamine.

TEST	PERIOD											
	Pre-expt. control		Days of experiment									
			4		11		18		23		33	
Group	C	D	C	D	C	D	C	D	C	D	C	D
1. Grip strength	65	55	66	55	67	57	66	54	68	52	64	52
2. Back lift	186	138	190	139	196	146	198	136	189	139	191	142
3. Tapping speed, initial			69	67	67	60	69	60	71	60	72	65
4. Tapping speed, final			60	59	62	56	60	55	62	55	61	58
5. Gross reaction time			73	61	82	82	75	88	76	98		
6. Co-ordination I			28	26	20	32	18	43	17	42	10	27
7. Co-ordination II			158	150	83	188	88	271	97	261	54	149
8. Speed and co-ordination	74	76	75	78	76	72	79	69	78	70	83	76

became impossible. Wi developed progressive tachycardia in work after 10 days on the diet; the work he did initially with pulse rates of 142 to 146 per minute involved the rates of 160 to 167 per minute during the period from the twelfth to the seventeenth days.

The pulse rate following brief severe work also was less affected than might be expected. In the present experiment the pulse rates were counted for the periods 0 to 15, 60 to 75 and 120 to 135 seconds following a 90-second run on the treadmill at 9 miles per hour and 8.6 per cent grade. The results are summarized in figure 5. The changes were small in all cases but the deficient subjects G, Wi, S and Ja all showed consistent increases at 60 to 75 seconds after 14 days and at 120 to 135 seconds after 33 days. Obviously the vitamin restriction had relatively only small effects on the functions concerned in this measurement.

The Harvard Fatigue Laboratory Fitness Test involves both pulse rate and presumed capacity to continue brief severe work. The scores in this test are listed in table 9. It is clear that the men who had been on an adequate diet for the preceding 6 months were unchanged at all times as appraised by this

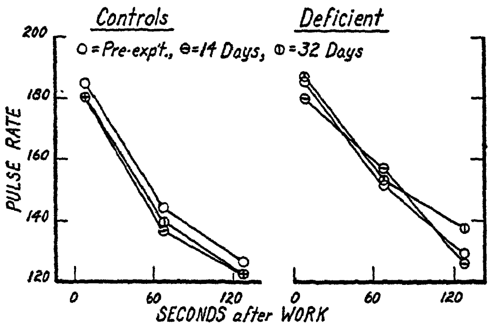

Fig. 5

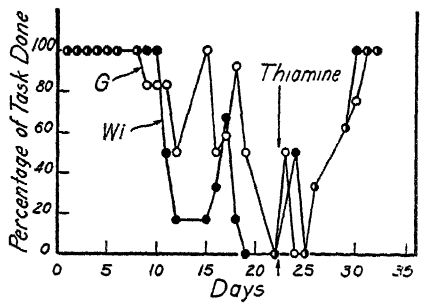

Fig. 6

Fig. 5. Average pulse rates following standard "anerobic" work for the "controls" (N, Jo, Wa, and T) and for the "placebos" (deficient subjects G, Wi, S and Ja) at the start and at 14 and at 32 days on the regimen.

Fig. 6. "Endurance" of subjects G and Wi as indicated by the percentage of the daily treadmill work completed. Note that all other subjects completed the full task every day.

test. Subjects G and Wi showed a rapid and progressive deterioration until thiamine was administered. An interesting result was the apparent improvement in the first week shown by Wa and T who had previously been on a diet judged just under adequacy.

The performance in the prescribed daily exercise can be used as an indication of capacity to endure moderately hard work. All the men (including S and also Ja until he was removed), except G and Wi regularly completed this work at all times. The performance of subjects G and Wi is depicted in figure 6. The declines in apparent endurance of these men after the first 8 days are striking as are their recoveries associated with thiamine supplementation alone.

The general result of these tests and measurements seems to be that only the endurance element of "fitness" was markedly altered and that only the combination of present total deficiency with prolonged previous slight inadequacy produced striking changes within the period of this experiment.

Speed and co-ordination. The average results from the psychomotor tests of speed and co-ordination are summarized in table 10. In general, all of these

measures showed slight to marked deterioration in the deficient group in comparison with the controls. These effects were visible in G and Wi by the 11th day of the experiment and tended to reversal after 10 days of thiamine supplementation. Both S and Ja resisted deterioration for about a week longer.

Maximal tapping speed remained very constant in the control group at all times so that the validity of the small but consistent reductions in the deficient group was clearly established. The choice-reaction-time for gross body movement increased in both groups but the increase in the deficient group was much larger than in the controls. The pipe-and-ball test which demands both a combination of speed and co-ordination more clearly differentiated the 2 groups, the controls showing a moderate practice improvement while the deficient men deteriorated and then, after thiamine, returned to about the initial level.

The pure co-ordination test (pattern tracing) very sharply differentiated the 2 groups. The scores in table 10 are not strictly comparable with those in the longer previous period (table 8 in part I) because of apparatus changes which made the task more difficult and required an adjustment of the work method on the part of the subject. In the present experiment the controls demonstrated a good learning capacity and improved steadily throughout. The deficient subjects not only did not gain through practice but showed a progressive deterioration until thiamine supplementation reversed the trend.

Subjective and personal appraisals. Self-rating and man-by-man rating questionnaires covered subjective states and general behavior. These were used in the present experiment in the same way as in the preceding prolonged period (cf. part I, table 10). In contrast with the stability and normality previously exhibited, the deficient subjects showed a sharp rise in subjective complaints and reported deviations from normal. In terms of the scoring system used the average subjective abnormality score for the deficient men rose from 2.0 on the fourth day to 12.3 on the eleventh, 12.5 on the eighteenth and 17.3 on the twenty-third day. After 10 days of thiamine supplementation the score fell to 4.0. The respective average scores for the control group at the same times were 1.5, 1.8, 1.8, 2.8 and 2.5. Man-by-man ratings, in which each subject appraised the relative behavior changes in each of the other subjects, gave closely similar results and checked with the appraisals by members of the Staff.

Within the deficient group the 3 subjects, G, Wi and S, could not be differentiated on the basis of the subjective self-ratings. Subject Ja showed no real changes in either rating up until the time he was withdrawn from the experiment (after 18 days). In terms of ratings of behavior by their fellows and by the Staff subjects G and Wi at all times were definitely considered to be much more affected by the regimen than were subjects S and Ja.

Personality and emotional adjustment. The application of the Minnesota Multiphasic Personality Inventory on the twenty-second day of the experiment revealed significant alterations in status of all the deficient subjects. The major points are summarized in table 11. Scores of over 70 in this test are considered to be related to a relatively significant deviation in the direction of the psychiatric characteristics listed. The very high scores for hypochondriasis, for depres-

sion and for hysteria are especially notable. In contrast, the controls showed only minor changes and these are readily explained by the tension created by the general rigidity and severity of the experimental regimen.

The group Rorschach test (Harrower-Erickson and Steiner, 1944) gave results which are interesting but did not reveal any consistent effects of the vitamin deficiency. The same was true of total performance in R. B. Cattell's Cursive Miniature Situation Personality Test (Cattell, 1941). However, the deficient subjects showed a distinct rise in the emotionality score in this test and this diminished after thiamine administration. All of the scores for the control subjects remained within narrow limits in Cattell's test. The studies on personality and emotion will be presented in detail elsewhere.

The sensory functions. Auditory acuity was repeatedly tested with each ear separately, making 3 ascending threshold determinations for each of 11 sound

TABLE 11

Standard scores in the Minnesota Multiphasic Personality Inventory on the scales: Hypochondriasis (Hs), Depression (D), Hysteria (Hy), Psychopathic Deviation (Pd), Paranoia (Pa), Psychasthenia (Ps), Schizophrenia (Sc), and Hypomania (Ma).

Normal average for each scale = 50, standard deviation = 10 . Averages for the men in the deficient and in the control groups just before and after 22 days in the present experiment

GROUP AND PERIOD	Hs	D	Hy	Pd	Pa	Pt	Sc	Ma
Deficient, before	46	58	55	54	55	54	57	60
Deficient, after	80	89	83	68	63	67	69	61
Control, before	45	52	59	45	53	47	45	46
Control, after	58	62	69	49	55	47	48	47

frequencies. No significant changes in hearing were found at any frequency at any time in any of the subjects.

Flicker fusion frequency (Simonson and Enzer, 1941; Brozek and Keys, 1944) and perceptual fluctuation (the geometrical interpretation of drawings of ambiguous cubes), both of which probably emphasize the cortical element of visual experience, showed small but reasonably consistent reductions in the deficient subjects and in none of the controls.

Measurement of the extent of the angioscotomata (paracentral extensions of the blind spot) with the stereocampimeter (Evans, 1938) showed pronounced effects of the vitamin deficiency. In terms of relative area the angioscotomata in subjects G and Wi were increased almost 3-fold in 2 weeks while subjects S and Ja, as well as the control subjects, showed no changes at any time. Just before thiamine administration (day 23) the scotomata attained still larger sizes in G and S but were quickly restored to normal thereafter.

Vibratory sensitivity was estimated in each subject on 5 occasions during this experiment by application of the bone-conduction unit of the audiometer ("Maico" model D5) to the external malleolus and to a fixed spot on the sole

in the arch of the foot. The vibrator was applied with a pressure of 700±100 grams and a frequency of 128 double vibrations per second. None of the subjects exhibited any change in threshold in either location at any time.

Sensitivity of the calf muscles to deep pressure has been stated to increase early in thiamine deficiency (Williams and others, 1940; Jolliffe, 1942; Barborka and others, 1943). Attempts were made to estimate this both by digital trial by experienced neurologists and by a quantitative test in which the pressure was applied with a blood pressure cuff. Neither method gave consistent indications of really significant changes though 2 of the neurologists were of the opinion that subjects G and Wi actually had some slightly increased sensitivity to deep muscle pressure. These results and those from other sensory test methods will be discussed in detail elsewhere.

Intellective functions. An endeavor was made to obtain an objective appraisal of intellective functions and the effects, if any, of the vitamin restriction on them. Five of the primary mental abilities isolated by Thurstone (1941), were tested: 1, the *number* factor was appraised by a multiplication test; 2, the space factor, i.e., the ability to understand spatial relations, was tested by performance in the comparison of two-dimensional geometric figures; 3, *word fluency* was estimated from the recall of words beginning with a designated letter; 4, immediate *memory* was tested as the ability to memorize word-number combinations; 5, inductive *reasoning* was appraised as the ability to supply missing units in a series of letters arranged in complicated schemes. In addition, *perceptual speed* was tested by checking errors in pairs of supposedly identical numbers. *Attention,* defined as the ability to attend continuously to oral series of 3 digit numbers, was also measured. All these tests had been practiced so that performance in them at the start of the acute restriction was at plateau levels.

The results cannot be discussed in detail here but the most important finding was that these functions, in general, proved remarkably stable in the face of the vitamin deficiency. Only in spatial manipulation and in number ability was there a consistent decline in the deficient group and even in these tests the changes were small. These latter functions tended to return to the previous control level of performance after a few days of thiamine supplementation alone.

Discussion. The present experiment suffers from several limitations, the most serious being the small number of subjects. The results might have been more apparently impressive if half of the men had not been controls but a true control group was essential. It could not be known, beforehand, that the several functions and appetite *would* remain normal and reasonably constant in the control situation. It was fortunate that a high degree of consistency characterized the results with the different individuals in the several groups. In general the findings with subjects G and Wi were closely similar and the rate and severity of their deterioration clearly differentiated them from subjects S and Ja. Further, these 4 men were well differentiated from the 4 controls, all of whom behaved reasonably consistently.

It was not expected that anorexia and nausea would dominate the symptoms as they did. It must be emphasized that after the first week or two the food

intake of the deficient subjects was maintained only by persuasion and the determination of the men to co-operate to the fullest extent. Apparently normal men ordinarily would never self-induce precisely this form of acute vitamin deficiency because voluntary starvation would intervene. In the present experiment it is important to distinguish the deterioration resulting from partial starvation alone from that due directly to the vitamin lack. While we have no exact parallel control on this point we do have experience from numerous other experiments with pure starvation. From such comparisons it can be affirmed that the changes in function observed here must be ascribed at least in very large part to the vitamin deficiency alone.

The utility and sensitivity of the special test procedures used here were well demonstrated. Aside from nausea, the deficiency observed must be considered largely "sub-clinical" because the most careful clinical examinations failed to reveal any really positive abnormalities besides the small peripheral nerve involvement as indicated by borderline changes in sensation in the lower extremities. It should be noted that even these rather dubious changes probably would have been considered insignificant if the neurologists had not made control examinations on the same men before and after their deficiency. The complete absence of oral, cutaneous and corneal changes is important.

There is no doubt that the previous dietary regimen affected the onset and severity of the symptoms. In this regard there is good support for the conclusion as to slight inadequacy of the low diet used for 6 months prior to the present experiment. It is notable that the 2 men (S and Ja) whose previous diet had been reasonably good were able to maintain essential constancy and normality of all functions, including capacity for hard work, for at least 2 weeks on the very low vitamin intake. This would seem again to refute the arguments of Jolliffe *et al.* (1939), of Johnson *et al.* (1942) and of Archdeacon and Murlin (1944) for the appearance of symptoms and physical debility in a very few days on diets supplying at least 10 to 30 times as much of the B vitamins as provided here (cf. Keys, Henschel, Taylor, Mickelsen and Brozek, 1944).

One of the unexpected findings was the relative stability of pyruvic acid metabolism. Though changes did occur these were much less than might have been expected from some reports (Lu, 1939; Platt and Lu, 1939). Marked changes in the lactate:pyruvate ratio (cf. Stotz and Bessey, 1942) were absent. In addition to the blood data discussed above, pyruvic acid in the 24-hour urine was measured every second day. The pyruvic acid excretion stayed within the previous normal limits (about 20 to 40 mgm. daily) in all men for the first 14 days and in N, Jo, Wa and T for the entire 32 days. In the third week, however, the values for G and Wi rose, respectively, to 147 and 56 mgm. Subject Ja showed no rise until his withdrawal from the experiment after 18 days while subject S showed a rise only to a maximum of 42 mgm. on the twenty-second day. In these acute conditions it appears that abnormality in pyruvic acid metabolism is by no means one of the earliest signs of deficiency. We may recall the report of Shils, Day and McCollum (1941).

It is interesting to list the major functions or areas of performance in the order

in which deterioration appeared. It should be noted that this order was approximately the same for all of the deficient men:

First—gastrointestinal (anorexia and nausea).

Early—emotional (depression, etc.), psychomotor (co-ordination), "fitness" as estimated from willingness to continue severe work.

Late—metabolic (lactic and pyruvic acid), neurological (including peripheral sensation), cardiovascular (heart rate during and after exertion), endurance (moderate to fairly severe work).

Very late or resistant—strength, general body chemistry, liver function, special senses, intellective functions, riboflavin and F_2 excretion.

All of the evidence here tends to indicate that the deficiency in thiamine overwhelmingly dominated the picture and no real deterioration was discovered to be related to the deficiency in the rest of the B complex. Only in this way can we explain the very clear and even dramatic improvement in all of the areas of debility which was produced by thiamine supplementation while continuing the same diet deficient in the other B vitamins.

It may be of interest to record here that at the end of the 33 days on this regimen all of the subjects returned to a good diet where they were able to indulge their wishes for foods withheld for some eight months—meat, milk, eggs, fresh fruits and vegetables. So far as could be discovered all the men were fully restored to normal within about 3 weeks and no residues could be discerned. With one exception, subject N, all of the men immediately volunteered again for similar experiments.

Performance-capacity tests were extensively used in this study and several tests, particularly those involving muscular co-ordination and effort, appeared to be useful indicators of certain aspects of "fitness" and the deterioration resulting from deprivation of the B vitamins. Several other research groups have relied very heavily on "maximal" performance tests in appraising vitamin effects (Johnson and others, 1942; Barborka, Foltz and Ivy, 1943; Archdeacon and Murlin, 1944). Since the validity of all conclusions from such tests depends on the attendant conditions even more than on the methods it is necessary to make some comments here. A full discussion of this complex subject will be presented elsewhere.

The results in all performance-capacity tests depend both on *actual capacity* and *willingness* to exert that capacity. Optimal, or at least constant, motivation is fundamental but it is almost impossible to judge motivation by simple observation or questioning the subjects. In the present study confidence in the performance test results is strengthened by the following considerations: 1. The subjects did not appreciate their own nutritional status until differentiation between the several groups by the performance tests was well established. Even then several control subjects persisted in believing themselves deficient. 2. The subjects volunteered for the hardships of the experiment in full knowledge that their participation would be useless unless they continued maximal effort in the tests throughout. Realization of the requirements for making their contribution effective was prominent at all times. 3. Emotional stimulants such as

"pep talks", threats or promises were carefully avoided as were all suggestive influences including expressions of concern or sympathy. 4. The subjects were completely adjusted to the experimental conditions. In the course of 7 months prior to the present experiment they became habituated to taking performance tests in a context designed to produce a motivational "set" aiming at optimal performance. 5. Under constant conditions before and during this experiment the performance levels were satisfactorily constant. The changes shown by the deficient subjects were much larger than observed in the controls or in the months of previous testing. 6. Changes in performance in the deficient group were not general but appeared to be characteristic for specific performance functions. 7. The direction and progression of changes in performance level were regular, parallel to the progressive vitamin depletion and at least partly reversible by thiamine alone. Reasonable physiological interpretation was applicable.

The necessity for validating the evidence in something like the foregoing manner should be recognized for all researches employing performance-capacity tests and this is not obviated simply by procedural or apparatus variants. In the Harvard fitness test pulse rate and time factors have been combined in an effort to reduce the dominance of motivation. The pulse rate factor is useful in detecting grossly inadequate effort but in the range of fitness and motivation generally obtained in this laboratory we have not found the pulse rate factor to add importantly to the information provided by the time alone.

In the introduction to this paper 4 questions were asked. It would seem that significant progress was achieved in reducing the areas of uncertainty represented by each of these questions: 1. It can be affirmed that many of the quantitative methods of this laboratory are sensitive to real deficiency and that they are much more discriminating than ordinary clinical procedures. 2. The deficiency itself has been characterized quantitatively over a wide variety of functions. 3. There can be no doubt that prolonged subsistence at a level just less than adequate for all ordinary functions results in abnormally rapid deterioration on a subsequent very deficient regimen. 4. Finally, thiamine is by far the most important of the B vitamins in these very acute conditions.

There are obvious limitations to the conclusions which may be drawn safely from the present experiment. The results pertain strictly only to relatively normal disease-free young men. The vitamin deficiency of the diet far exceeded anything which may be expected to occur naturally. Finally, in spite of the efforts to encompass a large part of the whole of human functions, there remain considerable sectors not covered. We can say nothing about effects on resistance to secondary stresses other than presented in the tests themselves. Such unexplored stresses included heat, cold, intense emotions, infections, loss of sleep, anoxia, physical trauma and hemorrhage. Further, the present tests and measurements were inadequate in some of the special senses (taste and smell), in gastro-intestinal and renal functions. More evidence certainly would be desirable on endurance and prolonged attention. We have no data on the sexual and reproductive functions. All of these points are mentioned here not so much to point out the present limitations—which are readily apparent—as to em-

phasize the fact that until all of these factors are properly assayed any conclusions on vitamin requirements and total deficiency effects must be considered tentative. Clearly final answers will be long delayed. It is proper to insist, however, that there is little reason to believe more complete coverage will necessarily point to higher vitamin requirements or to more profound effects of deficiency.

Extensive discussion of the literature seems unnecessary here. Strictly comparable experiments have not hitherto been reported and in no studies elsewhere has there been attempted even relatively comparable completeness of quantitative objective evaluation and control. Increasing experience intensifies our conviction as to the extreme difficulty in evaluating "fitness" and overall function and the unreliability of both clinical impressions and the results of uncontrolled performance-capacity tests.

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SUMMARY

1. Eight normal young men subsisted for 33 days on a diet adequate except in B vitamins which were extremely limited—0.008 mgm. of thiamine, 0.013 mgm. of riboflavin and 0.1 mgm. of riboflavin per 1000 Calories. Daily capsules provided adequate B vitamins to 4 of the men; the other 4 men received placebos. The activity was set at 4000 Cal. daily.

2. Half of the men in each group had subsisted for 6 months previously on an adequate intake of all nutrients except B vitamins which were restricted to about one-third of the National Research Council Recommended Allowances. The other men had received the same diet plus adequate supplements of the B vitamins. Accordingly there were 2 men in each of 4 groups with reference to previous and present diets: restricted-deficient, control-deficient, restricted-control, control-control.

3. Anorexia, and later nausea and vomiting, began in the restricted-deficient men after about 8 days and progressed to almost complete inability to eat in 18 to 20 days. The control-deficient men showed the same changes with a lag of 5 or 6 days. After 23 days the men in both these groups were given daily

supplements of thiamine only with a rapid return of appetite and regression of other symptoms and functional abnormalities.

4. The 24-hour urinary excretion of thiamine fell to the vanishing point in 2 to 4 days in all the deficient men. The excretion of riboflavin in these men also fell abruptly but tended to stabilize in a week at levels considerably in excess of the intake. The excretion of F_2 showed no marked relation to the intake of niacin in any of the men.

5. Changes in blood chemistry in the deficient men were primarily increases in both lactic and pyruvic acids in rest, in work and after glucose ingestion. These changes were insignificant up until 2 weeks and even thereafter were slight. Gastric emptying and tests of liver function showed no significant changes. Basal metabolism was substantially constant.

6. The restricted-deficient men showed progressive and eventually marked deterioration in endurance, co-ordination and "fitness" with trifling or absent effects on strength, vision, hearing and speed. Similar results were obtained with the control-deficient men with a lag of about a week.

7. Simple muscle efficiency was unaltered but cardiovascular capacity and respiratory efficiency were reduced by the deficiency. There was a diminution of heart size but no important electrocardiographic alterations. All of the deficient men showed pronounced tachycardia in work but only one of them developed resting bradycardia.

8. Intellective functions were resistant to the deficiency but there were marked changes in personality toward apathy, depression and hypochondriasis.

9. Detailed clinical, opthalmological and neurological examinations were largely negative except for minor indications of neuropathy in the legs.

10. The control subjects exhibited satisfactory constancy of all variables and functions tested.

11. Thiamine was much the most important B vitamin in this acute restriction. The bodily stores of thiamine are effective for at most a few weeks under these conditions.

12. The present results confirm the conclusion that the restricted diet described in part I of this paper was less than adequate or optimum, at least in thiamine, but that such inadequacy represented only a loss of a few days in the margin of safety.

13. It is concluded that the quantitative objective methods for estimating function used in this laboratory are properly sensitive to vitamin deficiency and are suitable to characterize the effects of diet on fitness, performance-capacity and the functional state. Serious limitations in both sensitivity and reliability of clinical methods in acute deficiency of the B vitamins were demonstrated.

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FLOW AND pH CHANGE OF SUBMAXILLARY SALIVA ASSOCIATED WITH VARIATIONS IN ACID-BASE EQUILIBRIUM[1]

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That the hydrogen ion concentration within the secretory cell is an important factor in determining the response of the submaxillary gland stimulated by pilocarpine was suggested by Eddy (1929, 1930, 1931) in an extensive series of experiments relating salivary flow to acid-base equilibrium changes and to blood flow changes. A partial confirmation of this suggestion was accomplished by Brassfield (1936) while comparing the pH changes of saliva with those of blood during the administration of gaseous mixtures low in oxygen and during intravenous injection of cyanide. The initial effect of those procedures was an increase in the pH of both blood and saliva which was attributed to the lowered CO_2 tension as a consequence of the augmented pulmonary ventilation. The decreased acidity was accompanied by a decreased secretory rate. Following the initial effect, the salivary pH decreased, presumably as a result of the formation within the gland of acid metabolites caused by the impairment of oxidations. It was concluded that the increase in acidity within the gland induced an increase in saliva flow in spite of the lack of oxygen and the continued high blood pH.

The object of this study has been to enquire further into the effects of intracellular acidity as reflected by changes in saliva pH upon secretory rate and the probable mechanisms by which the hydrogen ion effects upon saliva flow are achieved.

Methods. The submaxillary gland of anesthetized dogs was stimulated continuously by a constant injection of pilocarpine; saliva flow was followed with a modified Gibbs drop recorder; gases were administered and injections made as previously described (Brassfield, 1936). pH determinations and continuous pH curves were made and recorded for both blood and saliva by means of glass electrodes. The continuous pH curves for both blood and saliva were corded manually as were the continuous curves for saliva pH in the previous udy.

Results. Figure 1 shows typical results obtained by overventilating an animal with room air by means of a respiration pump. Saliva flow is diminished 75 per cent. A marked decrease in the hydrogen ion concentration of the blood is indicated by the increase in pH from 7.33 to 7.70. The hydrogen ion concentration of saliva is decreased also but the change is small (0.03 pH).

Although on the record the pH increase in saliva appears to occur during recovery from the procedure, actually this change takes place during the period of

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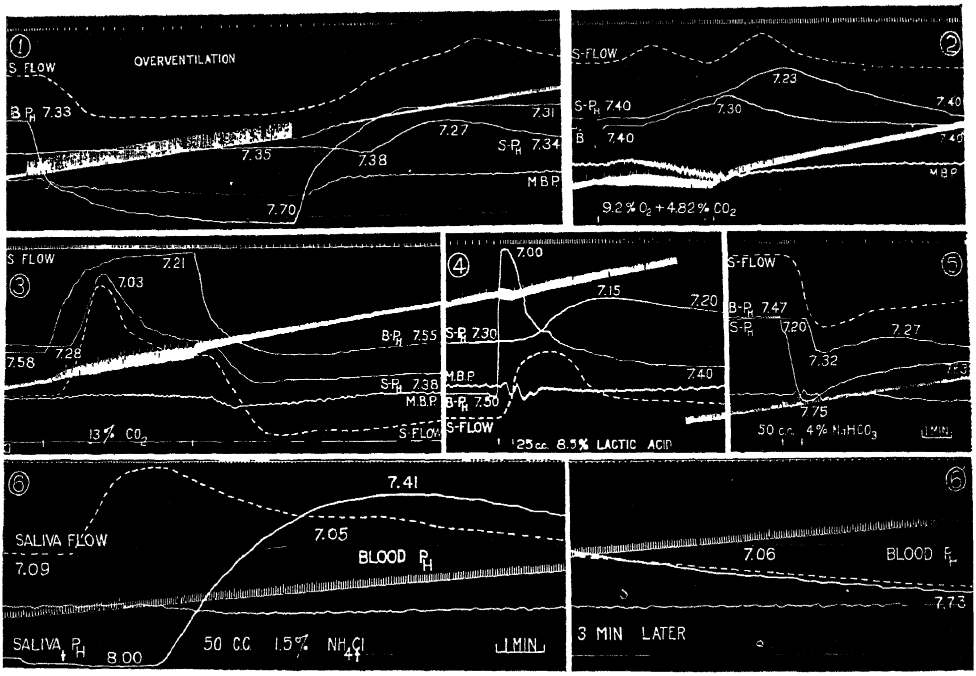
overventilation. This lag in recording is not due to any sluggishness on the part of the glass electrode, but to the capacity of the system consisting of the ducts, cannula and connections with the electrode. By the use of buffers injected into the duct at the hilus of the gland it was found that the lag consisted of at least 10 drops. Obviously there must be a few more drops contained in the collecting ducts.

The removal of CO_2 by the pulmonary overventilation accounts for the marked alkaline change in the blood. The lowering of the CO_2 tension of the blood in-



Figs. 1–6. Records of typical experiments showing responses to various procedures. The saliva pH curve has a lag equivalent to the flow of at least 10 drops of saliva. The broken line representing saliva flow is plotted from the drops indicated at the top of each record.

creases the CO_2 diffusion gradient between the gland cell and the blood, thereby providing a means for rapidly decreasing the CO_2 tension of saliva and thus increasing salivary pH. Since the salivary pH change is much smaller than the pH change of the arterial blood it is obvious that some other factor is also involved in determining the saliva pH. This is explained further in the discussion below.

On returning the animal to natural ventilation saliva flow and reaction exhibited a marked overshooting in the opposite direction but the blood pH showed only a small overshooting (0.02 pH). The marked overshooting of the saliva acidity as compared with that of the blood points to the increased gland acidity as being the chief cause of the enhanced salivary flow.

Having shown that artificial overventilation does decrease saliva flow and salivary acidity in a manner similar to the initial effects of low oxygen administration, and since it was inferred that the reduction of CO_2 was the causal factor in both cases, the next step in these experiments consisted in studying the effects of a gaseous mixture of carbon dioxide and low oxygen which would produce a CO_2 tension near that of the blood. Figure 2 illustrates the effects obtained by the administration of 9.2 per cent oxygen with a 4.83 per cent carbon dioxide. This will be seen to have caused only a decrease of 0.01 pH in the blood at the beginning of the procedure. Subsequently the acidity of both blood and saliva are increased but saliva shows the first and greater change if correction is made for the lag of the electrode. Saliva flow is increased 33 per cent at the beginning in contrast to the initial slowing previously observed when low oxygen alone is administered. A subsequent slowing of flow, however, takes place when the animal begins to show signs of respiratory and circulatory failure. On returning the animal to room air a further increase in acidity of the saliva is evident together with an increase of 50 per cent in flow while the pH of the blood shows an immediate inflection toward the preadministration value. These increases in flow are related to increased intracellular acidity rather than to the changes of the blood. The initial increase in flow accompanies the acid increase in saliva and precedes the blood change. Also, during recovery the flow is increasing while the blood is turning more alkaline.

From the alkaline effect observed in saliva as a result of blowing off CO_2 by overventilating with room air it was expected that the administration of air containing a high percentage of CO_2 would produce an acid change in saliva. Figure 3 shows that a high CO_2 mixture does produce an initial increase in the hydrogen ion concentration of the saliva as well as of the blood. But while the acidity of the blood continues to increase with rebreathing of the mixture that of the saliva decreases. On returning to room air the hydrogen ion concentration of the saliva decreases still further, although this decrease (0.14 pH) is less than the increase (0.24 pH) at the beginning of the high CO_2 administration. Secretory rate parallels the acid changes of the saliva. The highest rate of flow occurs at the beginning of the procedure where an increase of 180 per cent is observed.

Mixtures containing less than 8 per cent CO_2 usually produced an initial as as well as a late alkaline change in the saliva in contrast to the initial acid and late alkaline change occurring with the higher percentages. Secretion rate, however, increased during these administrations without showing the late decrease characteristic of the higher percentages. On returning to room air a further increase in alkalinity would occur accompanied by a decrease in saliva flow.

In contrast to these results, however, we have noted that the administration of carbon dioxide may produce parallel changes in the pH of both blood and saliva under two conditions: 1, after the gland has been stimulated by the continuous injection of pilocarpine for a period of three or four hours; 2, when the animal's blood pressure has fallen to a level around 60 mm. Hg. In these two cases the initial increase in saliva flow is much less than that observed under more favorable conditions and the accelerated flow is only transient.

Figure 4 shows a record typical of the effects of rapid intravenous injections of lactic acid. The blood is characterized by a rapid increase in acidity followed by a rapid decrease accompanying the increased pulmonary ventilation and the complete mixing of the injected acid with the blood. Recovery of the blood reaction then takes place rather slowly. Saliva becomes rapidly more acid and then gradually recovers. Secretion rate greatly increases immediately following the beginning of the injection, in this experiment 300 per cent, and then returns toward the preinjection rate, rapidly at first and then more slowly.

A small or slow injection of lactic acid, while always increasing the flow of saliva and the acidity of the blood, usually decreases the acidity of the saliva which is in keeping with the effects of administering gaseous mixtures containing carbon dioxide below 8 per cent.

Injecting sodium bicarbonate invariably produced a decrease in the acidity of the blood and a decrease in saliva flow, but the effects upon saliva pH were not uniform. Usually the pH of the saliva followed the directional changes of the blood pH as illustrated in figure 5. Sometimes, however, the saliva would show an initial increase in acidity followed by a decrease below the preinjection level. In some animals no change at all in salivary reaction could be observed as a result of bicarbonate injection.

Figure 6 illustrates the effects produced by the injection of a 1.5 per cent solution of ammonium chloride. No great increase in the acidity of the blood occurs as the decrease of only 0.04 pH indicates. The saliva, however, shows a marked increase in acidity as attested by the 0.59 pH decrease. The acid directional change in the saliva is accompanied or preceded by a 100 per cent increase in salivary flow.

Discussion. Lowering the alveolar CO_2 tension by artificial overventilation with room air produces effects in the pH of both blood and saliva similar to those occurring during the initial period of hyperpnea caused by the administration of gaseous mixtures low in oxygen. In neither procedure, however, is the change in saliva as great as in the blood. At least two possible explanations may be advanced to account for this difference. One is that saliva produced by pilocarpine has a CO_2 content about twice that of blood (de Beer and Wilson, 1932; McClanahan and Amberson, 1935) which would give the saliva greater buffering power against changes in CO_2 tension. However, preliminary experiments show (Brassfield and Behrman, 1937) that saliva elicited by pilocarpine has no greater buffering power against CO_2 than has blood. The other is that salivary content of sodium varies with the rate of secretion (Gregersen and Ingalls, 1931). Since there is a decreased rate of secretion in the two instances this would mean a decrease in the sodium content and thereby the total base, thus tending toward a more acid saliva, but the gland itself would be more alkaline. The sequence of events would be: alkaline blood, alkaline gland, reduced saliva flow, reduced saliva base, and increased saliva pH. The saliva reaction would then be the resultant of the effects of its reduced CO_2 tension and decreased content of base.

That the loss of CO_2 is a factor, however, in determining the increased pH of saliva during low oxygen administration is indicated in the experiments typified

by figure 2 in which CO_2 loss was prevented during low oxygen administration. The saliva now becomes more acid because of the accumulation of the acid metabolites resulting from the anaerobic metabolism. It is assumed that the gland also becomes more acid. If a decrease in the intracellular acidity of the gland either during the initial hyperpnea of low oxygen administration or during a period of overventilation with room air can decrease the flow of saliva, it seems reasonable to assume that the increased intracellular acidity occurring during the administration of the asphyxial mixture (fig. 2) is responsible for the increased saliva flow. The anaerobic metabolism alone is not capable of maintaining the secretion for long, but the increased acidity thus produced in the gland enhances secretion again when oxygen becomes available.

If CO_2 tension changes and varying amounts of base in the saliva with varying rates of secretion can account for the saliva pH changes during overventilation with room air, the pH changes in saliva during the administration of gaseous mixtures containing high CO_2 might be expected to have a similar explanation. The increased tension of arterial CO_2 thus produced would drive CO_2 into the tissues to make both the gland and the saliva more acid. But while the saliva turns acid at the beginning of the administration the increased salivary flow induced by the increased intracellular acidity in turn brings about the elimination of proportionally greater amounts of base. The increased amount of base in the saliva prevents as great a decrease in its pH as in the blood. The subsequent alkaline swing could be due to the further increased elimination of base as a consequence of the increased rate of secretion, and the alkaline change on returning to room air would be due primarily to the diminution of the CO_2 tension. The initial alkaline change in saliva produced by the lower CO_2 mixtures indicates that base is being eliminated from the gland in greater proportion to the amount of CO_2 entering the gland than is necessary to buffer the excess CO_2. The greater acid changes in the saliva from the fatigued gland may be taken as an indication of the inability of the secretory mechanism either to extract base from the blood or to liberate it from the gland.

The pH changes in saliva parallel those of blood following large injections of lactic acid and may be explained on the basis of an exchange of blood lactate for gland bicarbonate. This is in harmony with the results of Benetato and Oprisiu (1938) who found an increase in the lactates and acidity of parotid saliva of dogs immediately following exercise when the blood lactates were high. The rise in the pH of saliva following small or slow injections of lactic acid is in keeping with the results of Hug and Marenzi (1928), de Beer and Wilson (1932) and McClanahan and Amberson (1935) with hydrochloric acid. It is of interest to note that small or slow injections of acid produce similar effects upon saliva pH and flow as do administrations of low percentages of CO_2. It seems probable that a similar explanation holds for the saliva reaction in both procedures.

The variability of salivary pH changes following bicarbonate injection is in accord with findings in human saliva (Bloomfield and Huck, 1920; Starr, 1922; Mayr, 1931). Gesell and Hertzman (1926) showed that an acid swing occurred in cerebro-spinal fluid with rapid injections of bicarbonate which produced respir-

atory stimulation. Marshall and Nims (1940) found that the pH of the fluid on the surface of the cerebral cortex was sometimes acid and sometimes alkaline following injections of sodium bicarbonate. Benetato and Oprisiu (1938), however, found that parotid salivary pH paralleled that of blood with 4 per cent sodium bicarbonate injection.

The greater pH change in saliva than in blood with ammonium chloride injection suggests that ammonium chloride may be metabolized by the gland in a manner similar to that in the liver or that the ammonium radical stimulates the elaboration of acid substances. In whatever manner produced the acidity within the gland seems to be responsible for the increase in secretory rate.

Thus it is seen that variations in arterial pH produced by diverse acid-base equilibrium changes are not always accompanied by similar effects in salivary pH when the gland is stimulated continuously by pilocarpine. Induction of small variations of arterial pH may be accompanied by opposite changes in salivary pH. Variations in base with variations in rate of secretion, the type of metabolism within the gland cell and the reaction of the blood are all important factors in determining the reaction of saliva. However, an analysis of the sequence of events points to the importance of intracellular acidity in determining the rate of salivary flow, as was suggested earlier by Eddy (1929, 1930, 1931).

The suggestion has been made (Secker, 1934) that pilocarpine exerts its effect upon the submaxillary gland by liberating acetylcholine at the secretory nerve endings. List and Peet (1938) likewise suggest that the action of pilocarpine in sweat secretion is mediated through the nerve endings. Should this be confirmed the acid-humoral mechanism postulated by Gesell, Brassfield and Hamilton (1942) offers an explanation of the results obtained on the submaxillary gland continuously stimulated with pilocarpine. Integral parts of the acid-neurohumoral mechanism are intensity and duration of stimulation which are dependent upon the sum total of acetylcholine released at activated nerve terminals and the rate of destruction of this acetylcholine. Since the rate of destruction is inhibited by a decrease in pH, an increase in the acidity either on or within the gland cell should enhance the rate of secretion. This is in accord with the representative results illustrated in figures 1 to 6. These results harmonize also with those obtained on several cholinergic systems already studied: the central nervous system, the heart, respiratory muscle and the intestinal loop.

However, it must be pointed out that the effect of pilocarpine may not be mediated through the liberation of acetylcholine at the secretory nerve endings. Numerous investigations have shown that the response of the submaxillary gland to pilocarpine is different in some respects from that obtained either by direct nerve stimulation of acetylcholine injection (Demoor, 1912; Gregersen and Ingalls, 1939; Baxter, 1932; Pierce and Gregersen, 1937; Wills and Fenn, 1938; Brassfield and Hong, 1939; Wills, 1941; Brassfield and Hands, 1941). It is to be noted also that eserine fails to potentiate the action of pilocarpine on sweat secretion (Wilson, 1934). If pilocarpine does not act by liberating acetylcholine then some other explanation for the potentiating action of intracellular acidity must be sought.

SUMMARY

Overventilation in dogs receiving a continuous injection of pilocarpine produces an increase in the pH of both blood and saliva. These changes are attributed primarily to decreased CO_2 tensions. The change in salivary pH is smaller than the pH change in the arterial blood. This is thought to be due to the decrease in content of base resulting from a slower rate of secretion.

Administration of low O_2 in a mixture containing CO_2 approximating the tension of this gas in the arterial blood produces a greater decrease of pH in saliva than in arterial blood. Disturbances of the cellular metabolism induced by the impairment of oxidations contribute to this decrease in salivary pH. Since the increased salivary flow accompanies the decreased salivary pH but precedes the blood pH decrease, the increased secretion is attributed to the increased intracellular acidity.

The effect of administering air containing more than 8 per cent CO_2 is to produce initially a greater pH decrease in blood than saliva. After the initial decrease in saliva pH an increase is obtained and a further increase occurs on returning the animal to room air. With lower percentages of CO_2 only increases in salivary pH are produced.

Large injections of lactic acid produce parallel decreases in both blood and saliva but the change in saliva is less. Small or slow injections may produce an increase in salivary pH. Sodium bicarbonate injections usually produce an increase in saliva pH which is much less than the pH increase in the blood. Sometimes the pH of saliva shows an initial decrease.

Ammonium chloride injections produce much greater decreases in saliva pH than in blood.

Saliva flow changes are related to variations of intracellular acidity rather than to the reaction of either blood or saliva. A probable mechanism for the enhancement of flow by an increase in intraglandular acidity is suggested.

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CARBOHYDRATE AND ACETONE BODY METABOLISM OF LIVER SLICES AND THE EFFECT OF INSULIN

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The part played by insulin in the regulation of the metabolic function of the liver is poorly understood. It is well agreed that in the presence of diabetes insulin ameliorates the excessive catabolism of protein which accompanies the carbohydrate wastage. Bach and Holmes (1937) presented evidence that this effect could be due to direct suppression of hepatic gluconeogenesis from protein. They found that when insulin was added to preparations of liver slices the rate of accumulation of new carbohydrate was reduced. Stadie, Lukens and Zapp (1940), however, were unable to confirm this observation.

Another problem which deserves further study concerns the relation of insulin to the processes involved in the storage and release of liver glycogen. In the diabetic subject insulin produces a rise in liver glycogen, while in the nondiabetic animal the reverse effect is observed even though the blood sugar is maintained at hyperglycemic levels by the administration of glucose (Evans, 1941). The latter observation points toward the existence of a glycogenolytic effect which is exerted by insulin directly on the liver.

The present experiments were undertaken in order to study the effect of insulin on the turnover of both glycogen and total carbohydrate in liver slices obtained from normal and diabetic rats, and incubated under conditions approaching as nearly as possible a normal tissue environment. Observations on acetone body production were included.

METHODS. The animals were rats of Sprague Dawley strain and weighed 250 grams or more. There were two groups of normal animals. One was subjected to a preliminary 48 hour fast and the other allowed free access to Purina dog chow. A third group consisted of rats made diabetic by the subcutaneous injection of alloxan (150 mgm. per kilo). The latter animals were maintained on a diet containing 60 per cent carbohydrate, 10 per cent fat, and 25 per cent protein. The diabetes was controlled with daily doses of 2 to 4 units of protamine zinc insulin for 10 to 20 days until it was thought likely that kidney and liver damage induced by the alloxan had been repaired. Insulin was then withdrawn and the animals were used 3 days later when hyperglycemia and ketonemia had become well marked.

Rats were anesthetized with nembutal and exsanguinated by direct incision of the abdominal aorta. The liver was cooled to 5°C. until slices were prepared 15 to 20 minutes later. One cubic centimeter of serum, obtained from the rat's own blood, was placed in each of two small flat bottomed vessels which were designed to fit a standard Warburg manometer. A mixture of 95 per cent

oxygen and 5 per cent CO_2 was introduced and the vessels stoppered and weighed. Three liver slices approximately 0.3 mm. thick, weighing between 40 and 65 mgm., were cut free hand. Serum was used as a lubricant and the excess removed by touching the slice to filter paper. The slices were not floated in saline. Slices, differing in weight by less than 15 per cent, were placed in each of the two incubating vessels. Tissue weight was determined by the difference in weight of the vessel before and after introduction of the slice. The third slice, after being weighed, was dropped into a tube containing 1 cc. of normal H_2SO_4 for the determination of total fermentable carbohydrate. After hydrolysis at 100°C. for 2½ hours the contents of the tube were deproteinized with sodium tungstate. One aliquot of the centrifugate was analyzed for total reducing substances by the method of Somogyi (1937). Another was treated with washed yeast and the residual nonfermentable reducing substances determined.

To one of the incubating flasks was added 0.05 cc. of U 40 insulin (2 units) neutralized to pH 7.5[1]. The control flask received 0.05 cc. of water. After 2 hours of incubation at 37.5°C. the slices were removed, reweighed, and analyzed for total fermentable carbohydrate as described above. Total reducing substances, and in most cases fermentable carbohydrate, were determined on the original serum as well as on the serum in the flasks after incubation.

Serum acetone bodies were quantitated by the method of Shipley and Long (1938).

Results. *Glucose output by slices.* The amount of glucose yielded to the medium by a slice in the absence of added insulin was highest in the preparations obtained from diabetic animals and lowest in those from fasted normal animals. Fed normal animals were intermediate (table 1).

In the case of fed rats there was a very definite and consistent increase in glucose output when insulin was added to the medium. This increase varied from 62 per cent to 167 per cent and averaged 103 per cent (table 1). No consistent effect was obtained with slices from fasted normal animals.

In the diabetic group 4 out of 5 rats showed a decrease in glucose output in the presence of insulin. Rat 18 showed a slight increase. It should be noted that the latter animal differed from the other 4 in that the initial tissue carbohydrate content was relatively high.

Carbohydrate content of slices. With liver slices from fed rats insulin caused a distinct increase in the rate of breakdown of stored carbohydrate (table 1). The average carbohydrate content of the original tissue was 5.0 per cent. When slices were incubated without insulin the content dropped to 2.6 per cent, while in the presence of insulin the value fell to 1.1 per cent.

In the case of fasted rats and in most of the diabetic rats no effect of insulin was clearly evident (table 1). In one diabetic rat (no. 18) there was a definite lowering of carbohydrate content in the presence of insulin but this animal had shown a fairly high initial level. In all of the other instances of both fasted normal and fed diabetic rats the small glycogen store became rapidly exhausted

[1] Highly purified amorphous insulin powder containing 20 to 22 units per milligram was generously supplied by Dr. D. C. Hines of Eli Lilly & Co.

during incubation without insulin so that any possible tendency for insulin to accelerate the decline would not be demonstrable even if it existed. It was clear,

TABLE 1

Effect of insulin on glucose production and release of tissue carbohydrate

RAT NO.	GLUCOSE				TISSUE CARBOHYDRATE			
	Initial serum level	Produced by control slices (mgm. per 100 mgm. tissue)	Produced in presence of insulin	Per cent change due to insulin	Original content	Content after incubation —control	Content after incubation —insulin	Difference due to insulin
				Fed normal rats				
	mgm. %			%	%	%	%	
1	133	1.83	3.66	+100				
2	190	2.09	4.80	+130	6.9	3.9	1.8	−2.1 (54%)
3	160	1.99	5.31	+167				
4	171	3.11	5.30	+70	5.6	2.0	1.1	−0.9 (45%)
5	194	1.94	3.54	+82	3.5	2.1	0.8	−1.3 (62%)
6	87	1.48	3.70	+150				
7	121	1.22	3.07	+151				
8	120	2.12	3.92	+85				
9	133	1.53	2.43	+59				
10	121	2.46	3.98	+62	4.2	2.3	1.1	−1.2 (52%)
11	158	2.16	4.89	+126	4.7	2.7	0.9	−1.8 (67%)
Average.......		2.00	4.05	+103	5.0	2.6	1.1	−1.5 (58%)
				Fasted normal rats				
12	147	1.38	1.74	+26	1.4	0.6	0.7	+0.1
13	138	1.73	1.09	−37	1.1	0.5	0.6	+0.1
14	162	0.99	1.28	+29	0.9	0.4	0.0	−0.4
15	116	1.61	1.48	−8	0.8	0.1	0.4	+0.3
16	141	1.02	0.84	−18	0.4	0.1	0.1	0.0
Average.......		1.35	1.29	−4	0.9	0.3	0.3	0.0
				Fed diabetic rats				
17	610	1.77	1.48	−16	0.8	0.4	0.3	−0.1
18	539	4.24	4.96	+17	3.5	1.8	0.7	−1.1
19	517	3.02	2.29	−24	1.0	0.6	0.4	−0.2
20	550	2.93	2.44	−17	1.3	0.6	0.4	−0.2
21	983	2.59	1.40	−46	0.6	0.4	0.5	+0.1
Average.......		2.91	2.51	−14	1.4	0.7	0.5	−0.2

however, and rather disappointing that no increase in glycogen content could be demonstrated in the diabetic preparations after addition of insulin.

Synthesis and consumption of carbohydrate. In the control slices obtained from fed rats there were three instances of an over-all decrease of carbohydrate during

incubation (table 2). The other two preparations showed a very slight increase. In those instances where the controls showed a decrease the effect of insulin was to render this decrease less marked or actually to reverse the change so that the formation of new carbohydrate exceeded its breakdown (rats 2, 4 and 11). In the two instances in which carbohydrate synthesis was already predominating in the absence of added insulin the effect of the hormone was to increase the rate of accumulation (rats 5 and 10). Thus, in all five cases insulin promoted an

TABLE 2

Effect of insulin on the total carbohydrate present in the system: tissue + serum

	RAT NO.	CONTROL SLICE	INSULIN	CHANGE PRODUCED BY INSULIN
		mgm.	*mgm.*	*mgm.*
Fed normal rats	2	−1.70	−0.71	+0.99
	4	−1.05	+0.48	+1.53
	5	+0.17	+0.58	+0.41
	10	+0.20	+0.59	+0.39
	11	−0.32	+0.87	+1.19
Average.		−0.56	+0.36	+0.92
Fasted normal rats	12	+0.48	+0.65	+0.17
	13	+1.06	+0.47	−0.59
	14	+0.38	+0.35	−0.03
	15	+0.92	+1.00	+0.08
	16	+0.68	+0.54	−0.14
Average.		+0.70	+0.60	−0.10
Fed diabetic rats	17	+1.32	+0.94	−0.38
	18	+2.39	+1.96	−0.43
	19	+2.62	+1.64	−0.98
	20	+2.15	+1.53	−0.62
	21	+2.18	+0.56	−1.62
Average.		+2.13	+1.33	−0.80 (−38%)

The values are expressed in terms of milligrams of carbohydrate per 100 mgm. of wet tissue in 2 hours. Plus values denote formation of new carbohydrate; minus values disappearance of carbohydrate.

increase or lessened the tendency for a decrease in the carbohydrate content of the system.

The five control preparations from fasted rats all showed a moderate increase in total carbohydrate content during incubation (table 2). Insulin did not produce any consistent effect in either the magnitude or direction of the carbohydrate turnover.

The results with diabetic tissue were of considerable interest (table 2). In the first place it was observed that slices in the absence of added insulin were producing new carbohydrate at a much more rapid rate than it was being con-

sumed. Thus, the average carbohydrate synthesis by diabetic slices was 2.13 mgm. per 100 mgm. of tissue as compared to 0.70 mgm. for the "fasted normal" group and as contrasted with a consumption of carbohydrate of 0.56 mgm. by the "fed normal" series. The second important observation was that insulin invariably reduced the excessive accumulation of carbohydrate which occurred in the diabetic preparations The average reduction in the five preparations was 38 per cent. In rat 18, although the total carbohydrate production by the slice was reduced, the glucose output as mentioned above was increased. This may be attributed to the high initial glycogen content which provided an opportunity for insulin to stimulate glycogenolysis.

TABLE 3

Effect of insulin on acetone body production

	RAT NO.	INITIAL SERUM LEVEL	CONTROLS (GAMMA PER 100 MGM. TISSUE) PRODUCED	INSULIN TREATED-PRODUCED	PER CENT CHANGE DUE TO INSULIN
		mgm. %			
Fed normal rats	1	1.7	74	99	+32
	2	1.1	64	71	+11
	3	1.3	116	105	−9
	6	1.2	82	104	+27
	7	1.4	87	76	−13
	8	1.2	114	113	−1
	9	1.8	94	98	+4
	22	0.0	71	71	0
Fasted normal rats	12	11.7	105	55	−48
	13	6.2	157	161	+3
	14	3.8	125	123	−2
Fed diabetic rats	17	24.7	221	242	+9
	18	15.6	171	156	−9
	19	48.6	238	296	+24
	20	22.9	149	141	−5

Production of acetone bodies. Acetone body production was higher in fasted rats than in fed rats. This is in agreement with previous observations (Shipley, 1944). The output was extremely high in diabetic rats. In none of the three types of preparation was there any effect of insulin which was of sufficient consistency or magnitude to be considered significant.

DISCUSSION. The great bulk of labile hepatic carbohydrate consists of glycogen. A component composed of blood glucose and glycoprotein probably remains at a fixed low level. In the following discussion, therefore, it will be assumed that fluctuations in the glucose equivalent of total tissue carbohydrate will reflect changes in the glycogen content of the tissue.

In the present experiments liver slices, rich in glycogen, obtained from fed animals, showed a substantial and consistent increase in glucose output in the presence of insulin. There was a concomitant acceleration of glycogen breakdown. That the increased rate of glycogen disappearance was due to accelerated

breakdown rather than decreased rate of formation from noncarbohydrate sources is suggested by the finding that the calculations of carbohydrate balance offered no evidence that carbohydrate synthesis was decreased by insulin. In fact, the results were consistent with the reverse. One is thus justified in favoring the conclusion that insulin directly accelerated the conversion of glycogen to glucose. This conclusion is in complete agreement with the observations made on intact rats by Evans (1941).

In view of the fact that insulin promotes glycogen storage in the diabetic animal, one might expect the same to occur in the diabetic liver slice preparations. Such an effect could not be demonstrated, however. Other workers employing nondiabetic animals observed various effects on hepatic glycogen when insulin was added directly to isolated liver preparations. Lundsgaard, Nielson and Orskov (1936) found that one sample of crystalline insulin was without effect on the glycogen content of perfused cat liver while another produced glycogenolysis. Seckel (1938) observed decreased glycogenolysis in rat liver slices incubated in an artificial medium when insulin was added. Ostern, Herbert and Holmes (1939) in somewhat similar experiments, obtained the opposite effect with rabbit liver slices. In the perfusion experiments of Corey and Britton (1941) the addition of insulin resulted in an increased rate of glycogen breakdown in cat liver. If it is assumed that insulin plays the part of an enzyme in the glycogen $\rightleftarrows$ glucose equilibrium the facilitation of the reaction could be in either direction depending upon the conditions involved. The pertinent conclusion remains, however, that insulin does appear to exert a direct influence on this equilibrium.

It is possible to draw certain conclusions concerning the effect of insulin on the total carbohydrate balance of the system under study. This balance depends upon the relative rates of new carbohydrate formation and carbohydrate destruction. In fed normal rats insulin invariably promoted an increase in the accumulation of new carbohydrate in the preparation. This effect could be due to increased gluconeogenesis; however, other possible explanations are a decreased rate of carbohydrate oxidation or a decreased conversion to noncarbohydate compounds. None of these changes is compatible with the supposed action of insulin when it is administered to a diabetic subject. Here again the paradoxical results might be due to the existence of a reversible reaction.

Compatible with the latter proposal is the observation that in diabetic preparations insulin produced an effect which was completely the reverse of that shown in slices from normal fed animals. In diabetic preparations there was a decrease in the accumulation of carbohydrate in the system when insulin was added. This effect of insulin is the same as that observed by Bach and Holmes (1937) although these workers obtained their positive results with slices obtained from normal fasted rats. In the present experiments, preparations of the latter category failed to respond to insulin.

It should be noted that in the diabetic preparations carbohydrate accumulation in the absence of insulin was progressing at a very rapid rate compared with normal preparations, and that the effect of insulin was to reduce this rate. Our results are therefore consistent with the hypothesis that in diabetes there is an increased hepatic production of new carbohydrate and that insulin serves to

suppress this over-production. The data do not actually establish the validity of the hypothesis, however, inasmuch as changes in the rate of carbohydrate removal could give similar results. Bach and Holmes, however, could demonstrate no increase in either oxygen consumption or in R.Q. accompanying the decrease in rate of carbohydrate accumulation induced by insulin. Moreover, they observed a concommitant decrease in urea formation, and were therefore of the opinion that insulin suppressed gluconeogenesis from protein.

If one assumes that the present findings support the contention that insulin directly suppresses an excessive hepatic gluconeogenesis which occurs in diabetes it seems clear that the effect is not attained by the creation of a glycogen barrier in the liver cells. The effects were observed in preparations in which the glycogen content was not only low but actually in a course of further decline.

The effect of insulin on acetone body production merits little comment other than that no influence could be demonstrated under the conditions of the experiments. It would appear, therefore, that the observed changes in glycogen breakdown and carbohydrate turnover were not intimately coupled with the reactions comprising the ketogenic mechanism. In previous experiments relating to hepatic ketogenesis there was likewise no demonstrable correlation between alterations in ketogenesis and glucose metabolism. It was observed that anterior pituitary extract stimulated the acetone body output of liver slices without affecting the glucose production (Shipley, 1944).

SUMMARY

Insulin affected both the rate of glycogenolysis and the rate of accumulation of new carbohydrate when it was added to preparations of rat liver slices incubated in the rats' own serum.

When the initial glycogen content was high insulin accelerated glycogenolysis irrespective of whether the rat was normal or diabetic. No stimulation of glycogen deposition could be demonstrated in any preparation.

In slices of liver from normal fed animals insulin increased the rate of accumulation of new carbohydrate while in diabetic preparations this rate, already excessive, was consistently decreased by insulin.

The addition of insulin did not influence the rate of acetone body production in normal or diabetic preparations.

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A COMPARISON OF INTRAVENOUS AND ORAL VITAMIN TOLERANCE TESTS[1]

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There are few more controversial aspects of the general problem of nutritional assessment than vitamin tolerance tests, as is witnessed by a lack of general agreement concerning the most desirable mode of administration, the proper dosages, the best times for collecting specimens, or the interpretation of results. It was our intent to investigate 3 different points. First, comparison was made between oral and intravenous test doses of ascorbic acid, riboflavin and thiamine. Second, the effect of each vitamin on the urinary excretion of the others was determined. Finally a mathematical formulation was obtained for the urinary excretion following both intravenous and oral test doses. The scope of the work was limited to well fed young men, and it is emphasized that the results and conclusions are not necessarily applicable to deficient subjects.

METHODS. The observations were made on a group of 16 young men living for 16 days for the most part indoors in a temperate environment. Activities included laboratory work and football or marching almost every afternoon, with an estimated average daily work expenditure of about 3300 Cals. Their diet provided a considerable variety of items but an essentially constant intake of nutrients, as shown in table 1 for each meal and for the whole day. Breakfast consisted of white bread, butter, marmalade and coffee; dinner of bread, butter, jam, ½ pint of milk, a meat or egg product and prepared breakfast cereal; and supper of bread, butter, jam, ½ pint of milk, coffee, a meat or egg product, a cooked vegetable, ice cream and 1 large orange. Meals were eaten at 8:15 a.m., 12 noon and 5 p.m. Extra bread, butter, jam, tea and coffee were allowed in the evening.

Vitamin load tests were administered at approximately 8 a.m. For oral dosage, the vitamins were dissolved in 100 ml. of water. Intravenous doses were in sterile aqueous solutions. Urine was collected at the desired times in paper cups and the vitamins were stabilized with oxalic acid (8). Ascorbic acid was estimated by titration against 2:6-dichlorophenolindophenol (1); and riboflavin, thiamine and N^1-methylnicotinamide by fluorometric methods (5).

RESULTS. *A. Comparison of oral and intravenous tests.* The time relations for oral and intravenous tests are shown in figures 1, 2 and 3. In examining the charts it should be noted that the ordinates represent the total excretion after a given time and hence the slope of the curve is the measure of the rate

[1] This work was financed in part under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the President and Fellows of Harvard College.

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of excretion. When the curves for the test subjects become parallel with those for the controls, the effects of the dose of vitamins are finished. By these criteria, the effects of intravenous doses of thiamine, riboflavin and ascorbic acid are no longer apparent after 4 hours; and the effects of oral doses of the same vitamins are finished in 12, 6 and 8 hours respectively.

TABLE 1

Daily diet of 16 subjects

NUTRIENT	MEAL				DAY'S TOTAL
	Breakfast	Dinner	Supper	Night	
Calories	730	1360	1340	280	3710
Protein, grams	7	43	51	2	103
Ascorbic acid, mgm.	0	5	55	0	60
Thiamin, mgm.	0.2	0.7	0.5	0.1	1.5
Riboflavin, mgm.	0.1	1.0	1.1	0	2.2
Niacin, mgm.	1.6	6.9	9.1	0.6	18.2

TABLE 2

Lack of influence of combinations of vitamins upon urinary excretion of others

(Each figure represents the average for 3 different subjects)

COMBINATION	DOSE	ROUTE	EXCRETION IN 10 HOURS AFTER DOSE		
			Thiamine	Riboflavin	Ascorbic acid
	mgm.		*mcg.*	*mcg.*	*mgm.*
B_1, B_2	5, 3	Oral	580	1880	8
B_1, B_2	5, 1.5	IV	1000	1200	9
Controls	0		200	365	11
B_1, C	5, 250	Oral	420	430	95
B_1, C	5, 250	IV	1200	350	100
Controls	0		100	375	9
B_2, C	3, 250	Oral	180	1615	113
B_2, C	1.5, 250	IV	115	1000	108
Controls	0		195	365	10
Nicotinamide	50	Oral	90	385	14
Controls	0		90	300	10

As is well known, the rate of excretion is for a time higher and the total percentage of the test dose excreted is larger when a given dose of a vitamin is given intravenously than after oral administration. In the present experiments, the percentages of injected thiamine, riboflavin and ascorbic acid recovered in the urine were approximately 15 per cent, 40 per cent, and 20 per cent respectively; and of the same vitamins taken orally were 10 per cent, 35 per cent and 10 per cent respectively.

B. Lack of influence of individual vitamins upon the urinary excretion of others.

In the rat, thiamine deficiency results in a diminution of the tissue stores of ascorbic acid (11) and an increased excretion of riboflavin (10); and ariboflavinosis

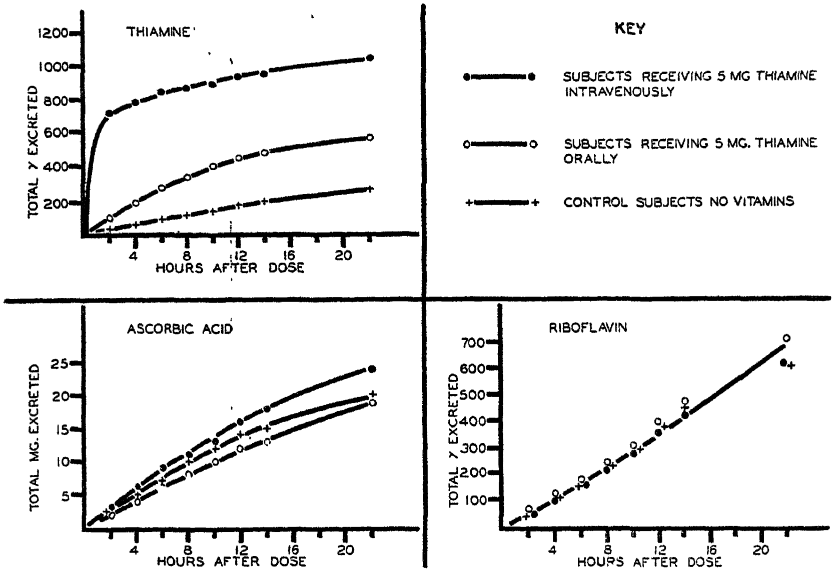

Fig. 1. Urinary excretion of thiamine, ascorbic acid and riboflavin after test doses of thiamine hydrochloride intravenously or orally. Curves fitted by inspection. Averages for 7 intravenous, 6 oral and 3 control subjects.

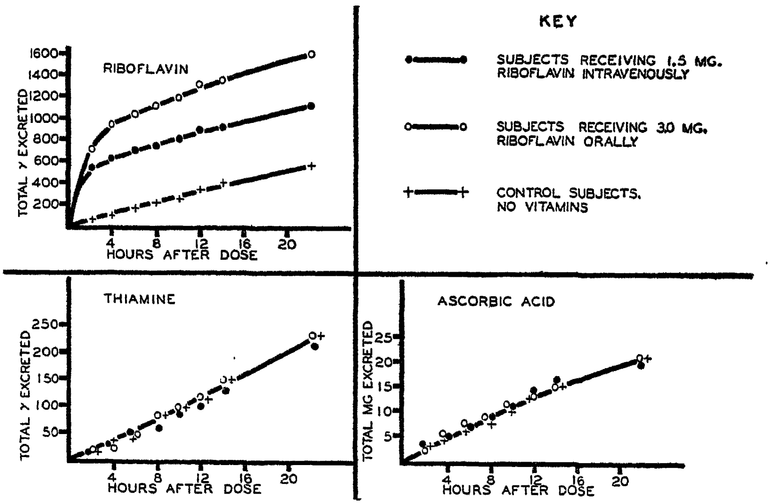

Fig. 2. Urinary excretion of riboflavin, thiamine and ascorbic acid after test doses of riboflavin intravenously or orally. Curves fitted by inspection. Averages for 7 intravenous, 6 oral and 3 control subjects.

results in depletion of tissue ascorbic acid without effect on thiamine. In the case of well fed subjects one could speculate that large doses of one vitamin might affect the urinary excretion of others, but this idea receives no support from

figures 1, 2, 3 and table 2. By comparison of the control with the test subjects, it is clear that doses of ascorbic acid had no significant effect on the excretion of thiamine or riboflavin; riboflavin, none on thiamine or ascorbic acid; thiamine, none on riboflavin or ascorbic acid; and nicotinamide, none on thiamine, riboflavin or ascorbic acid. Combinations of any 2 of the vitamins thiamine, riboflavin or ascorbic acid had no significant effect on the third.

The practical conclusion from these observations is that tolerance tests using mixtures of vitamins give results fully as reliable in well fed subjects as tests that use the vitamins individually.

C. Mathematical formulation of tolerance curves. Inspection of figures 1, 2 and 3 shows that any tolerance curve is compounded of 2 components, the

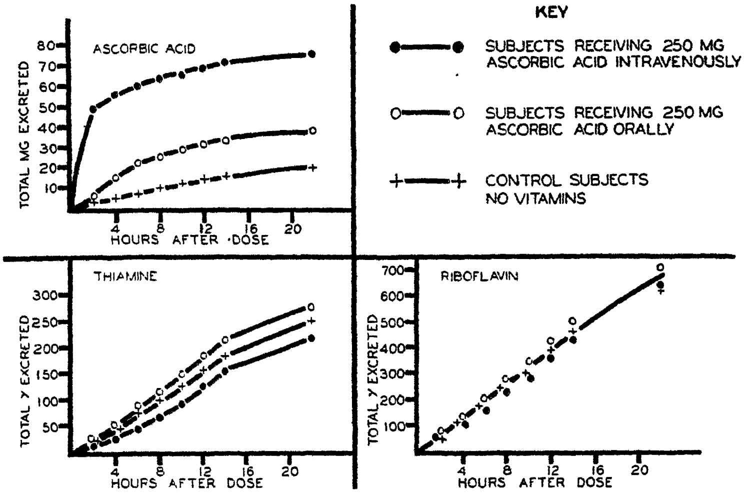

Fig. 3. Urinary excretion of ascorbic acid, thiamine and riboflavin after intravenous or oral test doses of ascorbic acid. Curves fitted by inspection. Averages for 7 intravenous, 6 oral and 3 control subjects.

first a linear representing the steady excretion when no vitamins are administered and the second a curvilinear representing the excess excretion due to the test dose. These will be taken up in the order control curves, intravenous curves and finally, oral curves.

A reasonable theoretical formulation for the various types of curve can be made by considering the mutual relations of serum levels, renal clearance and, after oral dosage, intestinal absorption. We shall treat in detail the case of ascorbic acid.

Control curves: ascorbic acid. For subjects not receiving test doses the serum level remains essentially constant throughout the day, or shows at the most a slight diurnal increase (fig. 4, controls), i.e.,

$$y_s = c \tag{1}$$

y_s representing the concentration of ascorbic acid in the serum and c a constant. If we assume a constant renal threshold for ascorbic acid and a reasonably

high rate of urinary flow, the rate of excretion of ascorbic acid is directly proportional to the difference between the serum level and the renal threshold. This difference is a constant and

$$y = at \tag{2}$$

where y is the total excretion at any time after observation begins, a is a constant and t the time in hours. Figure 4 shows the essential constancy of the serum level and the linearity of the excretion of ascorbic acid by control subjects.

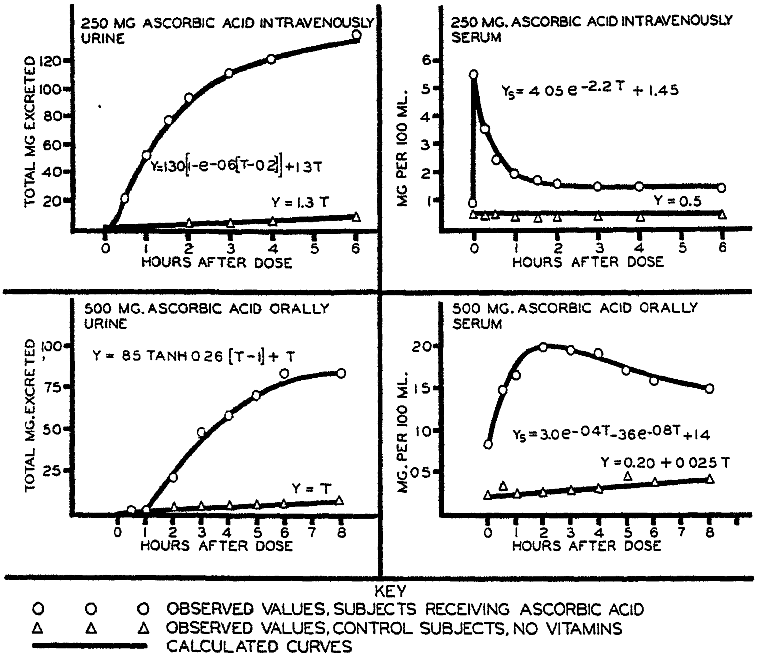

Fig. 4. Serum levels and urinary excretion of ascorbic acid compared with calculated curves derived in text. Subject L. C. received vitamins intravenously; subject R. W., orally; subjects A. R. and J. P. were controls for L. C. and R. W. respectively.

Intravenous curves: ascorbic acid. For subjects receiving test doses intravenously, the serum level is maximal at time O and then falls rapidly. Assuming metabolism, distribution throughout the body and renal clearance to be directly proportional to the increment in the serum, the rate of decrease of the serum level after a test dose is represented by the equation

$$dy_s/dt = -k(y_s - b)$$

where y_s is the serum level at time t, k has the dimensions of a velocity constant and b is the asymptote which the curve approaches.

Integrating, $ln\ (y - b) = -kt + a'$ and

$$y_s = a\ e^{-kt} + b \tag{3}$$

where b and k have the same meaning as above and a represents the maximal increase of the serum level above the asymptote. Good fit between observed and calculated values may be seen in figure 4. This simple exponential formulation has been used by Margaria, Edwards and Dill (6) for blood lactate; by Fishberg (2) for unnatural sugars in the blood; by Greville (3) for calcium tolerance tests; and has been thoroughly discussed by Greville (4) for intravenous glucose tolerance tests.

The rate of urinary excretion of ascorbic acid after an intravenous dose may be assumed to be directly proportional to the difference between the serum level and the renal threshold, i.e.,

$$dy/dt = f \text{ (serum level } - \text{ renal threshold)}$$

where y is the total excretion in time t and f is a constant. Substituting y_s from equation 3 and assuming the renal threshold to be constant, g,

$$dy/dt = f(a\,e^{-kt} + b - g), \text{ and simplifying}$$
$$dy/dt = h\,e^{-kt} + j, \text{ where } h \text{ and } j \text{ are constants.}$$

Integrating, $y = -h/k\,e^{-kt} + jt + m$ and combining terms $y = m(l - n\,e^{-kt}) + jt$. Reassigning conventional values to the constants and expressing the equation in a convenient form,

$$y = a[l - e^{-k(t - b)}] + ct \qquad (4)$$

where y is the excretion of the vitamin in t hours; a is the total excess of the vitamin excreted at $t = \infty$; k has the dimensions of a velocity constant; and b is a small constant related to the lag between the time of injection and the first measurable increase in rate of urinary excretion. For physiological purposes, when $(t - b)$ is negative it is considered to be O. The expression ct represents the linear excretion when no vitamins are administered. Good agreement between observed and calculated values after intravenous injection may be seen in figure 4.

Curves like equation 4 are familiar in studies on renal physiology, an example being the excretion of phenol red after intravenous injection (9). From the present data for ascorbic acid in urine and plasma in the control subjects (fig. 4) the minute renal clearance of ascorbic acid, UV/P, is of the order of magnitude of 5 ml. per min. which suggests an active tubular reabsorption of ascorbic acid. The tolerance test data allow estimates to be made at high plasma levels; these show minute clearances of about 30 ml. per min., further evidence of active tubular reabsorption.

Oral curves: ascorbic acid. The rather complicated picture presented by the serum level after oral test doses may be formulated by considering the serum level at any time t to be increasing in direct proportion to the amount of vitamin absorbed from the gastrointestinal tract and at the same time to be decreasing in direct proportion to the serum level. The rate of absorption from the gastrointestinal tract may be considered directly proportional to the amount of vitamin present, or

$$d\,GI/dt = -k'GI,$$

where GI represents the amount of vitamin in the gastrointestinal tract at time t, and k has the dimensions of a velocity constant. Integrating,

$$GI = a'e^{-k't} + b' = a'e^{-k't}$$

where a' represents the maximal amount present after the test dose and b' is in this case O since all of the vitamin is essentially absorbed. The serum level is being decreased according to the exponential curve represented by equation 2, $a\,e^{-kt} + b$. The combined effects on the serum level of gastrointestinal absorption and of simultaneous decrease according to equation 2 may be expressed thus:

$$dy_s/dt = c(k'a'e^{-k't}) - f(k\,a\,e^{-kt})$$

where c and f are different velocity constants.
Integrating, $y_s = -c\,a'e^{-k't} + f\,a\,e^{-kt} + g$
and expressing in orthodox fashion

$$y_s = a\,e^{-kt} - b\,e^{-k't} + c \tag{5}$$

where y_s is the serum level at time t; a, b and c are different constants such that $(a - b + c)$ is the serum level before the test dose; c is the asymptote which the curve approaches; and k and k' are two different velocity constants. Reasonably good fit between observed values and values calculated by the double exponential equation 5 is seen in figure 4.

The theoretical equation for urinary excretion after an oral test dose may be derived on the assumption that the rate of urinary excretion at any time, t, is directly proportional to the difference between serum level, according to equation 5, and the renal threshold; i.e.,

$$dy/dt = f\,(a\,e^{-kt} - b\,e^{-k't} + c - r) = f(a\,e^{-kt} - b\,e^{-k't} + c')$$

where y is the total excretion in t hours; a, b, and c are from equation 5; f has the dimensions of a velocity constant; and r is the constant renal threshold. Integrating,

$$y = \frac{fae^{-kt}}{-k} - \frac{fb}{-k'}e^{-k't} + fc't + g$$

Simplifying and expressing the constants in orthodox manner

$$y = a\,(l - b\,e^{-kt} + c\,e^{-k't}) + gt \tag{6}$$

For purposes of computation it is convenient to express equation 6 in terms of hyperbolic functions as follows:

$$tanh\ nt = \frac{e^{nt} - e^{-nt}}{e^{nt} + e^{-nt}}$$

Expanding as an infinite series

$$tanh\ nt = 1 - 2\,e^{-2nt} + 2\,e^{-4nt} - 2\,e^{-6nt} + \ldots.$$

In the present set of data, the arithmetical values for equation 6 reveal b and c to be approximately equal; and k' to equal $2k$. Hence, neglecting the higher powers in the infinite series above, equation 6 may be expressed to a first approximation as

$$y = a \tanh nt + ct \tag{7}$$

Correcting for the time between administration of the test dose and the first increase in rate of urinary excretion, equation 7 becomes

$$y = a \tanh n\,(t - b) + ct \tag{8}$$

where y is the total excretion in t hours; a is the total excess excretion of the vitamin over the control level when t is very large; n has the dimensions of a velocity constant; b is a constant related to the lag between ingestion of the test

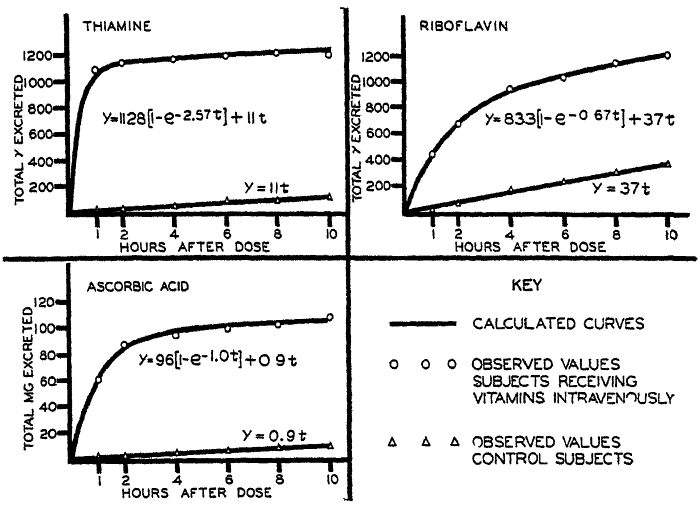

Fig. 5. Observed values compared with calculated curves derived in text. Averages for 7 subjects receiving vitamins intravenously and 3 controls.

dose and the first measurable increase in rate of excretion; and ct represents the linear rate of excretion if no test dose were given. For the region where $(t - b)$ is negative, y is assumed equal to ct. Reasonably good fit between observed and calculated values is seen in figure 4. Curves such as equation 8 are familiar in general renal physiology, an example being the rate of urinary flow following the ingestion of large doses of water (9).

Control, intravenous and oral curves: thiamine, riboflavin, ascorbic acid and N^1-methylnicotinamide. This section will deal exclusively with urinary findings. Control curves for thiamine, riboflavin, ascorbic acid and N^1-methylnicotinamide are fitted by equation 2, $y = at$ (figs. 5 and 6). The numerical value of a depends upon: the vitamin under study; the subject's state of saturation; and individual idiosyncrasy. The meals fed in the present experiments had surprisingly little effect on the linearity of the curves during the daytime hours, the hourly excretion of the vitamins being essentially constant.

Intravenous curves for thiamine, riboflavin and ascorbic acid are fitted by curves similar to equation 4, $y = a[1 - e^{-k(t-b)}] + ct$ (fig. 5). The constant b is small enough under most conditions to be neglected. Constants a and k are affected by the nature of the vitamin under study; by the subject's state of saturation; and by individual idiosyncrasy. In addition, a is in part determined by the size of the test dose. The expression ct is the linear component representing the excretion had there been no test dose.

Oral curves for thiamine, riboflavin, ascorbic acid and N^1-methylnicotinamide are fitted by equation 8, $y = a \tanh n(t - b) + ct$. The numerical values of the constants a and n are affected by the nature of the vitamin under study, by

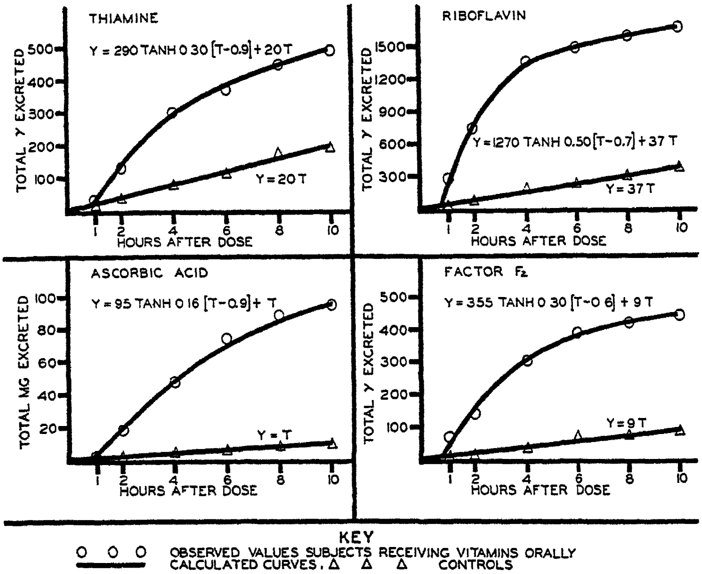

Fig. 6. Observed values compared with calculated curves derived in text. Averages for 6 subjects receiving vitamins orally and 3 controls. (The factor 1/20 should be used to convert F_2, expressed as microgram, quinine units, into N^1-methylnicotinamide, expressed as milligram.)

personal idiosyncrasy and by the state of the subject's saturation. In addition, a is dependent on the size of the test dose. The expression ct once more represents the excretion had there been no test dose. The constant b, related to the time between administration of the test dose and the first measurable increase in urinary excretion, is never small enough to be neglected.

Discussion. The reasonable theoretical explanation presented above for control, intravenous and oral curves allows certain practical suggestions to be made of interest to those engaged in routine surveys.

The fasting rate of excretion of thiamine, riboflavin and N^1-methylnicotinamide has been widely used as a measure of nutritional status in patients suspected of deficiency (7). This type of measurement is doubtless the surest way of avoiding the possible effects of meals. Even in well fed subjects it

may be taken as a measure of the steady rate of excretion during the day, being lower the more unsaturated the subject.

In both intravenous and oral curves the total excess excretion of the test dose (a of equations 4 and 8) and the velocity constants of the curve (k of equation 4 and n of equation 8) are physiologically, but not mathematically, related so that in general when one increases, so does the other. Likewise, the maximal rate of excretion (at $t = 0$ in the case of intravenous curves, figure 5; and at $t = 1$ hr. in the case of oral, fig. 6) increases when a and k or n increase. As a first approximation, the excretion in the first hour after an intravenous dose and in the second hour after an oral dose is a reasonably accurate measure of the maximal rate of excretion. It would be worthwhile to investigate the possibility of using in surveys these short periods rather than the longer times necessary to measure the total excess excretion. A possible invalidating factor is great individual variations which were not present in our subjects.

A possible clinical application of the curves presented above might be in detecting anomalies of absorption by a test run as follows: At zero time the subject empties the bladder and receives an intravenous injection of a mixture of vitamins. Urine is collected for 1 hour, this specimen providing an index of the effect of the intravenous dose. At 1 hour, the subject takes by mouth a mixture of vitamins. At 2 hours, the subject voids and the urine is discarded. By this time the effects of the intravenous dose are substantially finished. At 3 hours the subject voids again, this specimen providing an index of the effect of the oral dose. Comparison of the results for the intravenous and oral specimens might then disclose anomalies of intestinal absorption. This suggested test obviously would need a good deal of confirmation both with normal and with pathological subjects before its validity could be established.

SUMMARY

1. A comparison of intravenous and oral vitamin tolerance tests has been made with 16 subjects subsisting on the same constant diet in the same environment.

2. Any combination of ascorbic acid, riboflavin and thiamine could be administered without affecting the urinary excretion of other vitamins. Nicotinamide had no effect on the excretion of the above 3 vitamins.

3. The extra excretion after intravenous injection was complete in 4 hours; and after oral administration in 8 to 10 hours.

4. Mathematical formulation of the tolerance tests was accomplished. The control subjects, with no test doses, excreted the vitamins at a linear rate. Intravenous tests all fitted simple exponential curves. Oral tests all fitted somewhat more complicated double exponential curves, which were most conveniently expressed in terms of hyperbolic functions.

5. The above curves were all derivable on theoretical grounds from consideration of the blood levels as affected by metabolism and renal clearance, and, in the case of oral tests, intestinal absorption.

6. It is suggested for routine surveys that collection of urine for one hour

after an intravenous dose, and during the second hour after an oral dose, might yield results directly comparable with those obtained after collection periods of 4 and 10 hours respectively.

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HYPERTROPHY OF ADRENAL MEDULLA OF WHITE RATS IN CHRONIC THIOURACIL POISONING

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No reliable methods are available for determining slight degrees of hypertrophy of the chromaffin tissue because of its location. However, from great numbers of studies that have been made it is obvious that the adrenal medulla relatively has only a slight capacity for compensatory hypertrophy, and even in the most likely interrelation, namely, thyroid-adrenal medulla, although suspected for more than 40 years, only suggestive evidence of hypertrophy has been obtained by Tatum (1), Marine (2) in rabbits and by Goldberg (3) in sheep following thyroidectomy. There is also suggestive evidence that the medulla may undergo slight hypertrophy following hypophysectomy in dogs (unpublished). Recently another interrelation, adrenal cortex-medulla, has been suggested by the experiments of Vogt (4). She finds that infused epinephrine increases the output of cortical hormone in dogs and since the cortex is injured in thiouracil poisoning it is conceivable that the medulla might react with hypertrophy in an attempt to stimulate cortical function.

With the discovery by the MacKenzies (5) and by Richter and Clisby (6) of a new group of goitrogenic agents—sulfonamides and thioureas—which are not influenced by iodine medication and which act by inhibiting throxine production, a most intense thyroid insufficiency may be produced without immediate serious injury of other organs. This complete abolition of thyroxine production, heretofore unattainable, offers the severest test known for determining definitely whether the adrenal medulla undergoes compensatory hypertrophy or not.

In seven series of adult rats (175–300 grams) which had been given a daily dose of from 40 (0.5 per cent) to 120 (1.5 per cent) mgm. of thiouracil[1] in their food for one to four months, there was noted, in addition to marked thyroid enlargement, a progressive decrease in the weight of the adrenal glands. These adrenals were hyperemic and of a brownish or grayish brown color in contrast with the normal pinkish cream color. In a few isolated instances the adrenals were enlarged and contained grossly visible hemorrhagic cysts in their cortices.

The adrenal glands, after weighing, were fixed usually in 10 per cent formalin but occasionally one of the pair was fixed in Orth's fluid. Serial paraffin sections of one gland (usually the left) were made, and the section showing the greatest surface (approximately median) was used for a camera lucida outline of the medulla and cortex on graph paper with a magnification of 25 diameters. The squares covered by the medulla and cortex respectively were counted, reduced to square millimeters, and the percentage of the total surface occupied by medulla and cortex respectively was calculated. The pertinent average data of these

[1] We are indebted to the Lederle Laboratories for generous supplies of thiouracil.

seven series are give in table 1 and one experiment (no. 24) is tabulated in detail (table 2). All animals that died or were found to have pneumonia were excluded from the tables because of the hypertrophy of the cortex which occurs with infections. However, serial sections made of the glands from these infected rats also showed medulla hypertrophy. From table 1 it will be seen that the

TABLE 1

Average thiouracil effects

EXP. NO.	NO. OF RATS	SEX	WEIGHT			AREA MEDIAN SECTION		MEDULLA
			Body	Thyroid	Adrenal	Medulla	Cortex	
			gm.	*mgm.*	*mgm.*	*sq. mm.*	*sq. mm.*	*per cent*
10	6	F	229	127	33	1 55	3.92	28.2
10	5	M	324	144	32	1 28	3.51	26 7
14	5	F	247	170	33.5	1.96	4 63	30
15	6	F	196	185	21.7	1.40	3.22	30.3
15	4	M	244	314	30.6	1 52	4.17	26 8
17	3	F	237	129	32 3	2.28	3.96	36 7
22	13	F	255	54	32 8	1.45	4.30	26 7
24	8	F	175	67	27 4	1.31	4.03	30.5
25	10	M	191	49.8	22 7	1.31	3.33	28.2
Controls								
22	7	F	248	17.3	51.7	1.21	5.78	17.5

TABLE 2

(Expt. 24) Young adult females, 120 mgm. thiouracil daily

NO.	TREATMENT	WEIGHT			AREA MEDIAN SECTION		MEDULLA
		Body	Thyroid	Adrenal	Medulla	Cortex	
	days	*gm*	*mgm*	*mgm*	*sq. mm.*	*sq mm.*	*per cent*
a	14	168	32	37	1.68	5.05	25 0
b	30	208	56	33	1.17	3 80	23.5
c	46	165	52	23	1.29	3.91	24.8
d	60	163	79	23	1.75	4 80	26 7
e	74	188	79	39	2.44	4.20	36.7
f	90	156	74	18	1.43	3 00	32.3
g	115	186	90	24	2.26	3 76	37.5
h	115	167	75	22	2 10	3 76	35 8
Average...........		175	67	27.4	1.77	4.03	30.5

medulla area of rats receiving thiouracil varied from 26.7 per cent to 36.7 per cent of the total surface of the median section, while in control rats the medulla area of median sections averaged only 17.5 per cent of the total area. These percentages indicate only the size of the medulla in relation to the cortex and such percentage increases obviously may be due to hypertrophy of the medulla or shrinkage of the cortex or both.

Examining the data further one finds that the absolute surface of the medulla

in all experiments is significantly greater than that of the controls in rats receiving at least 80 mgm. daily for 14 or more days. We have estimated the medulla area of the largest section in many stock rats sacrificed for other reasons and the percentages of the total area occupied by medulla corresponded closely with the controls mentioned above, except that the percentage is higher in male rats because of the normally much larger cortex in the female.

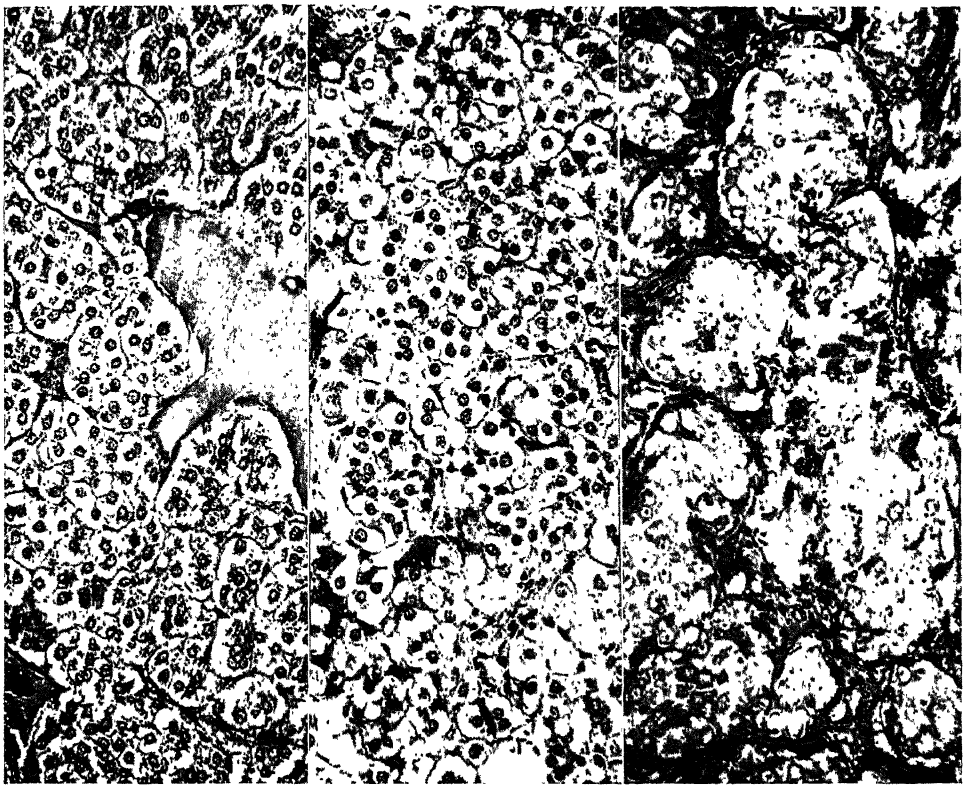

Fig. 1 Fig. 2 Fig. 3

Fig. 1 (Left). Normal adrenal medulla—adult male rat. × 200.

Fig. 2 (Center). Adrenal medulla—male adult rat (15j) fed 120 mgm. thiouracil daily for 5 months. × 200.

Fig. 3 (Right). Adrenal medulla—female adult rat (8a) fed 80 mgm. thiouracil daily for 130 days. × 200.

Experiment 24 (table 2) ran for 115 days—the rats being killed at intervals of approximately two weeks. All rats were young adult females and received 120 mgm. of thiouracil daily. The weight of the adrenal glands progressively decreased despite the progressive increase in the absolute area of the median medulla section. As will be brought out in another paper (9), this weight loss is due to the shrinkage of the cortex. The above data clearly indicate that with prolonged thiouracil feeding there is an easily recognizable hypertrophy of the medulla (figs. 1 and 2).

In another experiment not included in the above tabulations there was one rat which, because the medullae of the adrenals were so much more hypertrophic than those seen in any other experiment, is referred to separately. This rat, an old female, received 80 mgm. of thiouracil daily for 130 days and when sacrificed weighed 308 grams. There were anasarca, ascites, pleural effusions and enlarged dark red cervical, periaortic, axillary and inguinal lymph nodes, but no pneumonia. The spleen weighed 0.87 gram. The adrenals weighed 0.165 gram and the right contained a large hemorrhagic cyst in the cortex. The hypophysis weighed 0.033 gram, the largest we have encountered in thiouracil fed rats.

Microscopically there was marked hypertrophy of the medulla and involution of the cortex. The combined cortical areas of median sections (excluding the cyst) were 9.59 sq. mm., while the combined medullary areas of the same sections were 13.30. sq. mm. Hence the medulla occupied 58 per cent of the total surface of the median sections. The medulla cells had grown through to the capsule in both glands, completely destroying the cortex for nearly half the circumference of one gland and gave a suggestion of neoplasm (pheochromocytoma), but there was no invasion of the capsule. The remaining cortex was extremely hyperemic and greatly narrowed and the cortical zones were distorted. The cells of the cortex were small and highly vacuolated. The medulla cells were enlarged but no larger than glands showing lesser degrees of hypertrophy. The cells were polyhedral, arranged in nests and cords separated by a delicate stroma and very large irregular congested sinusoids. The cell membranes were distinct, the cytoplasm was abundant and stained a pale blue or purplish color with hematoxylin and eosin. The nuclei were large vesicular and the nucleoli prominent. An occasional mitotic figure was present (fig. 3).

The question arises whether these hypertrophic and hyperplastic medulla cells are secreting an excess of epinephrine, or whether like the hyperplastic thyroid cells their secretory activity is inhibited. All the glands fixed in Orth's fluid (relatively a poor solution for demonstrating the chromaffin reaction) took an intense chrome stain, much more than normal control rats' adrenals. The other adrenal gland in several instances was used for epinephrine determination, using the Folin-Cannon (7) method, and epinephrine stores as much as twice (500 mgm. per 100 grams) that present in normal adrenals (250 mgm. per 100 grams) were found. One can conclude that there is active epinephrine secretion in these hypertrophic medullae. Whether there is increased excretion of epinephrine has not been determined. It is somewhat paradoxical to see a high storage of hormone under conditions requiring increased secretion and excretion since usually under these conditions glands (thyroid, pancreas) show depletion of the store of hormone. However, it is of interest to note that very high stores of epinephrine have been found in human pheochromocytomas where the patient exhibits continuous symptoms of epinephrine poisoning (8), as well as in those cases where the symptoms were paroxysmal.

SUMMARY

Data are presented from a series of seven experiments comprising male and female adult rats which received from 40 to 120 mgm. of thiouracil daily in their

food from 14 to 385 days. These rats show an irregularly progressive increase in the size of the adrenal medulla. The hypertrophic medullae took a more intense chrome stain than normal adrenals and the epinephrine store as measured by the Folin-Cannon method was significantly greater than normal. It is suggested that the thyroid-adrenal medulla interrelation is a direct one.

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THE RIBOFLAVIN AND VITAMIN B_c POTENCY OF TISSUES FROM RATS FED SUCCINYL SULFATHIAZOLE WITH AND WITHOUT LIVER SUPPLEMENTS[1]

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It has been shown by several investigators that succinyl sulfathiazole retards the growth rate of rats which receive purified sucrose rations and that liver or liver concentrates counteract the depression in growth. The inclusion of succinyl sulfathiazole in purified rations decreases the amount of riboflavin excreted, particularly when lactose or dextrin diets are fed (1). It has also been observed (1) that rats gained in weight more rapidly when dextrin diets were fed than when sucrose or lactose diets were used. Because of these observations, and because most of the experiments with sulfa drugs have been carried out with purified sucrose rations, the present study was undertaken with dextrin diets.

EXPERIMENTAL. The dextrin basal ration consisted of casein (Labco) 24 per cent, dextrin 67 per cent, salts IV 4 per cent, corn oil 5 per cent, thiamine 250 μgm., pyridoxine 250 μgm., nicotinic acid 250 μgm., riboflavin 300 μgm., calcium pantothenate 2 mgm., choline 100 mgm., inositol 100 mgm. and biotin 1.5 μgm. per 100 grams. Weanling male rats from Sprague-Dawley were used and each rat received 1 drop of halibut liver oil per week. One per cent of succinyl sulfathiazole and 2 per cent or 6 per cent solubilized liver were added as supplements to this ration as indicated in table 1. The supplements were added at the expense of the entire ration. The low riboflavin diet was prepared by reducing the riboflavin supplement to 60 μgm. per 100 grams of ration. All rations were fed *ad libitum*. The average rates of growth per week from the 1st through the 6th week for the various groups are presented in table 1.

At the end of the 6th week, 4 of the 6 animals in each group were sacrificed by decapitation. The livers and tissues were prepared for analysis by the procedure used by Schweigert *et al.* (2). The riboflavin content of the samples was determined by the fluorometric method of Conner and Straub (3) with certain modifications, and the vitamin B_c potency of the samples was determined by the method of Teply and Elvehjem (4). Both *Streptococcus lactis* R and *Lactobacillus casei* were used as test organisms. The liver samples for B_c analysis

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were digested for 24 hours with takadiastase at pH 4.5 prior to assay. The riboflavin and B_c potencies of the tissues are presented in table 2.

DISCUSSION AND RESULTS. The addition of 1 per cent of succinyl sulfathiazole to the dextrin basal ration markedly inhibited the rate of growth in all groups where solubilized liver was not added. The retardation was very pronounced after the third week on experiment. The addition of 2 per cent solubilized liver counteracted this effect, which is similar to the results observed when sucrose diets are fed (5). The rate of growth was 33 grams per week when the basal ration was fed and increased slightly to 35 and 36 grams per week when solubilized liver was added. Rats which received riboflavin equivalent to that found in the solubilized liver (230 μgm. per gram) in addition to the basal diet grew at the same rate as the controls. The rats which received only 60 μgm. of riboflavin per 100 grams grew at an average rate of 16 grams per week and this was reduced to only 8 grams per week when 1 per cent succinyl sulfathiazole was added.

TABLE 1

Growth rate of rats which received succinyl sulfathiazole and solubilized liver

RATION	NUMBER OF ANIMALS	AVERAGE RATE OF GROWTH PER WEEK						
		1st	2nd	3rd	4th	5th	6th	Ave. for all 6 wks.
		gm.	gm.	gm.	gm.	gm.	gm.	gm.
Dextrin basal	12	27	35	37	33	35	29	33
Dextrin basal + 1.0 per cent S.S.	12	23	24	23	13	12	13	18
Dextrin basal + 2 per cent Sol. liver	6	29	42	43	39	36	29	36
Dextrin basal + 2 per cent Sol. liver + 1 per cent S.S.	6	30	40	39	35	38	26	35
Dextrin basal + riboflavin ≅ to that in 2 per cent Sol. liver	6	28	37	42	36	28	22	32
Dextrin basal + riboflavin ≅ to that in 2 per cent Sol. liver + 1 per cent S.S.	6	26	26	20	10	9	8	14
Dextrin basal + 6 per cent Sol. liver	3	21	42	50	32	44	27	36
Dextrin-low riboflavin basal	12	18	16	17	16	14	11	16
Dextrin-low riboflavin basal + 1.0 per cent S.S.	12	18	6	9	7	5	2	8

S.S. = Succinyl sulfathiazole.

The addition of 1 per cent of succinyl sulfathiazole had little effect on the riboflavin content of the livers and no effect on the riboflavin content of the muscle tissues either when 300 or 60 μgm. of riboflavin per 100 grams of ration were fed. It would seem that if riboflavin were the only limiting factor in the diet and any beneficial synthesis of riboflavin occurred when the dextrin low riboflavin diet was fed, the riboflavin content of the livers or tissues might be

considerably lower when succinyl sulfathiazole was added. The riboflavin content of the liver and muscle was not affected by the addition of 1 per cent of succinyl sulfathiazole, which would indicate that some other factor(s) was not synthesized in large enough amounts by the intestinal microbes and a slower rate of growth resulted.

TABLE 2

The effect of succinyl sulfathiazole and solubilized liver in the diet on the riboflavin and B_c potency of rat tissues

(All values expressed as micrograms per gram of fresh tissue)

RATION	NUMBER OF ANIMALS	RIBOFLAVIN				B_c POTENCY (S. LACTIS)		B_c POTENCY (L. CASEI)	
		Liver		Muscle		Liver		Liver	
		Range	Ave.	Range	Ave.	Range	Ave.	Range	Ave.
Dextrin basal	8	21.0–31.0	26.7	2.38–2.92	2.58	0.34–1.18	0.70	0.31–1.08	0.62
Dextrin basal + 1.0 per cent S.S.	8	18.4–31.6	23.6	2.30–2.95	2.54	0.18–0.48	0.29	0.12–0.42	0.28
Dextrin basal + 2 per cent Sol. liver	4	26.8–34.8	31.3	3.30–3.82	3.61	3.4 –6.4	5.1	3.1 –5.6	4.2
Dextrin basal + 2 per cent Sol. liver + 1 per cent S.S.	4	27.4–33.0	30.4	3.31–3.84	3.55	3.3 –4.5	3.9	3.0 –4.0	3.3
Dextrin basal + riboflavin ≅ to that in Sol. liver	4	33.1–36.8	34.8	3.23–3.33	3.30				
Dextrin basal + riboflavin ≅ to that in Sol. liver + 1.0 per cent S.S.	4	22.1–35.6	28.8	3.13–3.33	3.20				
Dextrin basal + 6 per cent Sol. liver	3	29.2–34.8	32.4	2.98–4.05	3.44	5.4 –7.0	6.0	5.6 –6.4	6.1
Dextrin-low riboflavin basal	8	8.2–13.5	10.5	1.01–1.30	1.21	0.61–1.18	0.90	0.55–1.30	0.87
Dextrin-low riboflavin basal + 1.0 per cent S.S.	8	5.3–11.8	8.9	1.02–1.65	1.31	0.18–0.36	0.26	0.14–0.36	0.26

S.S. = Succinyl sulfathiazole.

The addition of either 2 or 6 per cent solubilized liver, or riboflavin equivalent to that in solubilized liver, caused an increase in the concentration of riboflavin in both the liver and muscle tissue. The increase was most marked in the muscle tissue (from an average of 2.56 to 3.40 μgm./gram). The magnitude of the riboflavin values for these tissues is similar to that obtained in earlier work with sucrose diets and comparable riboflavin intakes (2). It is of interest to note that when 760 μgm. of riboflavin per 100 grams of ration were fed (either as pure riboflavin or in solubilized liver) an increase in the vitamin content of the

tissues was observed but the growth rates were approximately the same as were observed when 300 μgm./100 grams were fed.

A marked depression in the B_c potency of the livers was noted when succinyl sulfathiazole was added to the basal diets that contained 300 or 60 μgm. of riboflavin per 100 grams. Other workers (6) have observed similar effects when succinyl sulfathiazole was added to sucrose diets. The B_c potency of the livers from rats which received 60 μgm. of riboflavin per 100 grams was slightly higher than the B_c potency of livers from rats which received 300 μgm./100 grams. This is probably due to the limited rate of growth due to the subminimal riboflavin intake. The average B_c potency of the livers from 16 animals fed the basal diets was 0.75 μgm. per gram and 0.27 μgm. per gram when 1 per cent of succinyl sulfathiazole was added to these diets.

The B_c potency of the livers was markedly increased when either 2 or 6 per cent of solubilized liver was added to the basal diet which contained 300 μgm. of riboflavin per 100 grams (table 2). The liver fraction as fed contained an equivalent of 11.5 μgm. of B_c per gram as measured by *S. lactis* and 22.0 μgm. of B_c per gram as measured by *L. casei*. The addition of 1 per cent of succinyl sulfathiazole to the diet which contained 2 per cent solubilized liver did not appreciably lower the B_c potency of the livers. Apparently the drug did not affect the utilization of this factor.

In one experiment the B_c potency of the muscle tissues was determined after autolysis and subsequent takadiastase treatment. The muscle tissues from rats which received the basal diets averaged 0.23 μgm. per gram and 0.10 μgm. per gram from rats which were fed 1 per cent of succinyl sulfathiazole in addition to the basal diet (average of 8 animals in each group). In the other experiments where only takadiastase treatment was used, the values were less than 0.10 μgm. per gram of tissue and there was no appreciable difference in the B_c content of tissues from various groups. Work is now in progress to find better methods to liberate the "folic acid" from muscle tissues as well as other tissues. These data show that both the riboflavin and the B_c potency of the livers can be correlated with the dietary intake but the growth rate is not increased when larger amounts of riboflavin or "folic acid" sources are added to the basal diets.

SUMMARY

Rats which received 1 per cent of succinyl sulfathiazole in addition to the dextrin basal diet grew at a much slower rate than rats without the drug. The riboflavin content of the livers and muscle tissues was not affected by the ingestion of succinyl sulfathiazole but when either 2 per cent or 6 per cent of solubilized liver was fed, the riboflavin values were increased over those observed when 300 μgm. of riboflavin per 100 grams of diet were fed. The B_c potency of the livers from rats which received the succinyl sulfathiazole was markedly reduced as compared to the B_c potency of the livers from rats which received the basal diets, and the B_c potency of the livers was increased 5 to 9-fold by the inclusion of 2 or 6 per cent of solubilized liver in the diet.

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MECHANISMS OF CAROTID BODY STIMULATION[1]

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While the chief effects of carotid body stimulation have been clearly established, there is still confusion regarding the precise mechanism of this stimulation. Following the pioneer work of Heymans et al. (summarized by Heymans and Bouckaert, 1939) it has been generally confirmed and accepted that anoxemia is a powerful and an important normal stimulus initiating carotid body reflexes. Experimentally, other stimuli, including certain drugs, are also effective. A short discussion of these stimuli is included in a recent article by Dripps and Comroe (1944).

The drugs which have been used to produce carotid body reflexes may be divided into two general classes according to their mode of action (Dripps and Comroe, op. cit.; and others). One group, comprising the sulfides, cyanides, etc., apparently produce their effects by inhibiting intracellular respiratory enzymes, their action thus resulting in anoxemia of the affected cells. Von Euler, Liljestrand and Zotterman (1939) present evidence that the essential change occurring during anoxemia is one of increased intracellular acidity, thus supporting the previous conclusion of Winder (1937) and Bernthal and Weeks (1939) that an intracellular change in pH is a prime factor in normal carotid body stimulation.

The second group of drugs is characterized by the fact that its members, nicotine, lobeline, acetylcholine, etc., are synaptotropic agents. Heymans and Heymans (1927) apparently first demonstrated the action of this group upon the respiratory reflexes—an action which has been abundantly confirmed by other investigators. It is generally assumed that synaptotropic agents, when applied experimentally, produce their effects in exactly those loci in which such substances play a physiological rôle. The action of this group of drugs presumably indicates, therefore, that a cholinergic humoral agent is normally involved in the initiation of chemoreceptor reflexes.

The experiments of von Euler, Liljestrand and Zotterman (1939, 1941) have analyzed further the rôle of cholinergic substances in carotid body physiology; these writers have been able to show that while ammonia will block carotid body reflexes initiated by anoxemia or by drugs which affect the intracellular pH, it does not block the reflexes initiated by synaptotropic drugs. Their evidence indicates, therefore, that the site of action of cholinergic substances is not upon the receptor cell, but is rather somewhere along the afferent pathway. Their data further indicate that this point of action on the afferent pathway is within the carotid body: they have recorded potentials from the sinus nerve during

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cholinergic stimulation, and neither their degeneration experiments nor the earlier ones of de Castro (1926) will support the assumption that this nerve differs anatomically, between brain stem and carotid body, from other sensory nerves that are not affected by cholinergic substances. Von Euler et al. have described this point of action within the carotid body as a synapse, although their writings are somewhat vague as to the anatomy involved.

The evidence cited above indicates that a cholinergic substance is normally liberated in the carotid body during stimulation, and that this substance is involved in initiating impulses on the afferent limb of the reflex arc. The present work is an attempt to identify this cholinergic substance with acetylcholine.

Unfortunately, present methods are not sensitive enough to measure accurately the acetylcholine content of an organ the size of the cat carotid body, which weighs only about a milligram. However, a close correlation has long been known between the presence of acetylcholine and the enzyme that hydrolyzes it, cholinesterase (Nachmansohn, 1940). Accurate micro-methods are available for assaying cholinesterase activity (Glick, 1938; Sawyer, 1943). According to the studies of Mendel et al. (1943), there are two enzymes hydrolyzing acetylcholine: one, found in brain tissue, also hydrolyzes acetyl-beta-methylcholine (Merck's mecholyl), and has been designated as true cholinesterase; the other, obtained from blood serum and certain glands, also hydrolyzes benzoylcholine and is called pseudo-cholinesterase. Sawyer and Hollinshead (paper in press) have shown that in ganglionic synapses the locus of true cholinesterase, but not pseudo-cholinesterase, is identical to that of acetylcholine. Therefore, the presence of true cholinesterase is presumptive evidence of the presence of acetylcholine.

If the cholinergic substance involved in normal carotid body stimulation is acetylcholine, carotid body tissue should be rich also in true cholinesterase. In the present work, therefore, carotid bodies of the cat have been analyzed for this enzyme. The results indicate that acetylcholine is probably not the mediator involved.

Material and methods. Twelve cats were used in these experiments. Under ether anesthesia their thoraces were opened and their tissues freed of blood by perfusion through the heart with physiological saline. The carotid bifurcations were removed to dishes of unbuffered Ringer-Locke solution; the carotid bodies were then dissected out and trimmed of excess tissue under a binocular dissecting microscope, blotted on filter paper to remove surface fluid, and placed in tightly corked weighing tubes. After weighing, the samples were triturated with powdered quartz while still in the weighing tubes. One hundred twenty-one microliters of 30 per cent glycerine was added to the sample, and extraction was allowed to proceed for 16 to 20 hours, the tissue being stirred twice during that time. Subsequent treatment, but for the length of the digestion period, was the same as that accorded extracts of ganglia (Sawyer and Hollinshead, op. cit.) and will not be redescribed here. The duration of the digestion period for carotid bodies was five hours. Total cholinesterase activity was measured as before against acetylcholine chloride, and relative proportions of true and

pseudo-cholinesterase were determined by the use of their specific substrates, mecholyl and benzoylcholine chloride.[2]

The results are given in terms of cholinesterase coefficient, Q ChE, defined as the number of milligrams of acetylcholine hydrolyzed in one hour by 100 mgm. of tissue. As in the previous work the coefficients for mecholyl and benzoylcholine have been calculated in terms equivalent to acetylcholine, not in terms of their own molecular weights.

In preliminary experiments, the two carotid bodies from a cat were analyzed separately. However, it soon appeared that the enzyme activity of the tissue was so low that it was impossible to obtain from that weight of tissue adequate

TABLE 1

Cholinesterases in the cat carotid body

CAT	WEIGHT OF TWO CAROTID BODIES	Q_{ChE}				
		Q_{ACh}	Q_{Mech}	% of* ACh	Q_{Benz}	% of* ACh
	mgm.					
CA′	2.7	2.11	0.44	*21*	0.57	*27*
CA	0.8	1.69	0.59	*35*	1.39	*82*
CB	1.5	2.12	0.35	*17*	0.69	*33*
CC	2.2	3.43	1.23	*36*	0.99	*29*
CF	2.1	2.99	0.51	*17*	1.03	*34*
CG	2.3	0.91	0.42	*46*	0.45	*49*
CH	3.0	3.03	0.14	*5*	1.12	*37*
CI	2.1	1.30	0.46	*35*	0.32	*25*
CJ	3.0	1.65	0.26	*16*	0.46	*28*
CK	1.7	3.59	0.38	*11*	1.15	*32*
CL	1.9	2.81	0.69	*25*	0.83	*30*
CN	1.3	2.28	0.25	*11*	0.91	*40*

* The hydrolysis of mecholyl and benzoylcholine have been compared with ACh hydrolysis, assuming the latter to be 100%.

amounts of an extract sufficiently active for the complete determination; thereafter, the carotid bodies from one animal were combined to form a single sample.

RESULTS. The results of cholinesterase assays on the carotid bodies of twelve cats are listed in table 1. True cholinesterase activity (Q Mech) is almost invariably weaker than pseudo-cholinesterase activity (Q Benz). In fact, total acetylcholine hydrolysis, while dependent on both enzymes, is here much more closely a function of pseudo- than of true cholinesterase; the coefficient of correlation (r) between Q ACh and Q Benz values is 0.64; between Q ACh and Q Mech, r = 0.30. The interdependence of Q ACh and Q Benz is conclusive evidence that the carotid body enzyme hydrolyzing benzoylcholine is actually pseudo-cholinesterase rather than the benzoylcholinesterase recently described (Sawyer, in press).

[2] We are grateful to Dr. Frederick Bernheim and to Hoffman-La Roche Inc. for supplying the benzoylcholine chloride used in these assays.

Table 2 summarizes the data on cholinesterases in the carotid body, adrenal medulla, and various ganglia and nerve fibers. Many of these data are from a previous paper (Sawyer and Hollinshead, op. cit.). There are several facts which

TABLE 2

Cholinesterases in ganglia, nerves and related structures

ORGAN	ANIMAL	NUMBER OF ORGANS ASSAYED	AVERAGE WEIGHT USED IN ASSAY	Q_{ChE}				
				Q_{ACh}	Q_{Mech}	% of ACh	Q_{Benz}	% of ACh
			mgm.					
Carotid body...	cat	24*	2.0	2.33 ±0.246†	0.48 ±0.081	*21*	0.83 ±0.095	*36*
Adrenal medulla.......	cat	2	9.4	3.93 ±2.19	2.50 ±0.32	*64*	1.18 ±0.80	*30*
Ganglia								
Superior cervical sympathetic...	cat	20	8.6	28.4 ±1.78	7.98 ±0.521	*28*	7.65 ±0.601	*27*
	guinea pig	2	1.8	11.7 ±1.1	9.6 ±0.2	*82*	0.04 ±0.04	*0.3*
Coeliac.......	cat	3	9.4	19.6 ±2.44	3.31 ±0.178	*17*	5.94 ±0.819	*30*
	guinea pig	1	1.2	48.8	3.54	*7*	16.4	*33*
Sensory (7th dorsal root)	cat	2	14.8	4.52 ±0.28	0.73 ±0.07	*16*	1.70 ±0.05	*38*
Motor fibers								
Hypoglossal...	cat	1	9.7	0.49	0.31	*63*	0.04	*8*
Cervical sympthetic trunk	cat	15	5.0	3.69 ±4.00	2.48 ±0.234	*67*	0.52 ±0.108	*14*
Ventral root..	cat	2	18.6	0.46 ±0.05	0.34 ±0.0	*74*	0.08 ±0.005	*17*
Mixed fibers								
Vagus.........	cat	1	14.4	2.89	1.08	*37*	0.67	*23*
Sciatic........	cat	3	18.5	0.69 ±0.057	0.29 ±0.103	*42*	0.15 ±0.089	*22*
Sensory fibers								
Dorsal root...	cat	2	28.8	0.12 ±0.03	0.04 ±0.04	*33*	0.03 ±0.004	*25*
Femoral cutaneous...	cat	1	21.9	0.34	0.06	*18*	0.10	*30*

* The 24 carotid bodies were from the 12 cats listed in table 1. The two organs from each cat were assayed together.

† The standard errors were calculated according to the formula SE $= \sqrt{\frac{\Sigma d^2}{n(n-1)}}$.

should be emphasized. True cholinesterase activity in the carotid body is very low in comparison with its level in the adrenal medulla and ganglia containing synapses. The superior cervical ganglion contains the true cholinesterase sixteen to twenty times, and the adrenal medulla five times, as concentrated as in the carotid body. In fact, certain peripheral nerve fibers such as the vagus

and the cervical sympathetic trunk contain the enzyme in higher concentrations than does the carotid body. On the other hand, the true cholinesterase level in the non-synaptic sensory ganglion is of the same order of magnitude as in the carotid body. The absolute true cholinesterase activity of the more heavily myelinated nerve fibers, especially sensory, is low in comparison even with the carotid body.

The "% of ACh" columns have been included in the tables so that, in addition to their absolute activities, the relative amounts of true and pseudo-cholinesterase in the various organs may be compared. It can be seen that great variation exists in these relative activities among synaptic ganglia; the absolute true cholinesterase level is more nearly constant. Among fibers there is a tendency for the relative activity of true cholinesterase to be high in motor, intermediate in mixed, and low in sensory, while the relative amounts of pseudo-cholinesterase are lowest in motor, intermediate in mixed, and highest in sensory, fibers. The value for true cholinesterase in the dorsal root is not significant. The sensory ganglion and also the carotid body have much the same relative amounts of true and pseudo-cholinesterase as has the peripheral sensory nerve.

Discussion. In their otherwise illuminating papers upon the mechanism of carotid reflexes, von Euler, Liljestrand and Zotterman have been very vague in their discussions of the morphological elements involved in alterations in pH and the action of cholinergic substances. They have hypothecated a synapse along the afferent pathway, and inferred that ganglion cells, distinct from the physiological receptors, play a part in this synapse. These inferences seem unfortunate to the present writers, for their experiments are of greater significance when interpreted in terms of known anatomical facts.

Essentially, chemoreceptor tissue combines three elements: the first is a rich vascular supply, by which stimuli reach the reactive elements; the second is the chemoreceptor cell; the third is the abundant sensory nerve supply. The chemoreceptor cells have been variously, and no doubt confusingly, described as modified ganglion cells (Meijling, 1938), "paraganglionic tissue" (Goormaghtigh and Pannier, 1939) or simply as specialized sensory cells (Hollinshead, 1943). Whatever the name attached to them, however, it must be realized that these cells form the bulk of chemoreceptor tissue, and to them must be attributed the specific reactions of chemoreceptor tissue. They are not merely supporting cells for the inter-mingled sensory nerve fibers, for their cytological structure indicates physiological activity and high specialization (de Castro, 1926; Hollinshead, op. cit.). It is undoubtedly these specific cells which are affected by anoxemia—the changes in intracellular pH associated with normal and certain pharmacological stimuli of chemoreceptor reflexes occur within these cells. This has probably been assumed by most investigators, but has not always been clearly enunciated.

The origin and site of action of cholinergic substances has been still less clearly interpreted in morphological terms. The "ganglion cells" referred to by von Euler and his co-workers may be either of two elements, it is not clear which; either they are true ganglion cells, which are found occasionally in or within the

vicinity of chemoreceptor tissue, or they are the modified ganglion cells described by Meijling. True ganglion cells and their axons are not sufficiently numerous in chemoreceptor tissue to play the important rôle assigned ganglion cells by von Euler et al; in so far as they affect chemoreceptor function they are undoubtedly vasomotor elements as de Castro (op. cit.) indicated. The modified ganglion cells of Meijling are really the chemoreceptor cells themselves, a fact which has apparently not been appreciated by von Euler et al. Thus the physiological receptors and the ganglion cells of von Euler are the same things, and the synapse which they hypothecate must actually be the point of contact between the chemoreceptor cells and the third element of chemoreceptor tissue, the sensory nerve endings.

Meijling has interpreted this point of contact as having the properties of a synapse, although he has described morphological continuity between the sensory nerve fibers and the chemoreceptor cells. According to most authors, (de Castro, op. cit.; Nonidez, 1935, 1937; Goormaghtigh and Pannier, op. cit.; Hollinshead, 1939, 1940) these endings lie in close apposition to, but not within, the chemoreceptor cells. Von Euler and his co-workers have rejected the idea of a direct action of cholinergic substances upon these endings, because sensory nerve endings are not, as a rule, so stimulated. However, as Dripps and Comroe (op. cit.) also point out, many sensory fibers, including the pressoreceptors from the carotid sinus, would not be expected to respond to chemical stimuli, for they are normally affected by mechanical distortion of their immediate surroundings. In showing that the action of cholinergic substances is not upon the chemoreceptor cell, von Euler et al. have actually proven that this action is upon the sensory nerves; these are the only additional morphological elements in the afferent path. Whether the point of contact between these sensory endings and the chemoreceptor cells should be termed a synapse appears immaterial.

The work of von Euler and his co-workers indicates, therefore, that the normal mechanism of carotid body stimulation involves two phases: the first is a change of pH in the chemoreceptor cell; the second is a liberation of a cholinergic substance to affect the sensory endings. The only apparent source of this cholinergic substance is the chemoreceptor cell.

For a microchemical analysis of the chemoreceptor cell, as undertaken in the present work, the carotid body of the cat is an especially favorable specimen. It is a compact, rounded body about a millimeter in diameter, and can easily be isolated under the dissecting microscope. In contrast to the carotid body of dog and man, in which there are scattered cell groups or lobules separated by abundant connective tissue, the cat carotid body is primarily cellular (fig. 1) with few connective tissue elements, although it is, of course, abundantly vascular. Since the results of analysis for the enzymes are usually stated in terms of the hydrolyzing power per unit weight of tissue, this homogeneity is obviously important.

In a previous paper (Sawyer and Hollinshead, op. cit.) the true cholinesterase content of tissues has been shown to be closely correlated, as a rule, with the known acetylcholine content of those tissues, while the pseudo-cholinesterase

bears no direct relation to the presence of acetylcholine. Inspection of table 2 will reveal that the true cholinesterase content of sympathetic ganglia of the cat and guinea pig, and the adrenal medulla of the cat—that is, those places in which acetylcholine is known to be liberated upon nerve stimulation—is high, in contrast to the relatively low concentration found in dorsal root ganglia. The carotid body contains even less true cholinesterase than dorsal root ganglia, and therefore presumably no physiologically significant concentration of acetylcholine.

If the carotid body cell-sensory nerve fiber relationship were a synapse employing acetylcholine as the transmitter, then true cholinesterase should be produced by the carotid body cells, and if these latter elements were the source of the

Fig. 1

Fig. 2

Fig. 1. Section of a carotid body of a cat, stained with acid fuchsin and Masson's aniline blue mixture. The numerous cell groups are separated from each other by blood vessels primarily; the whole forms a compact organ. × 85, as reproduced.

Fig. 2. Section of a carotid body of a cat showing the numerous nerve fibers within it. Pyridine-silver stain. × 85, as reproduced.

cholinesterase found here, the connective tissue and the blood vessels must obviously be regarded as dilutents of these. In the adrenal medulla, however, the nerve fibers and endings, the sources of its cholinesterase activity, are insignificant in bulk per unit weight of gland, yet its cholinesterase value is high. Since in the carotid body cholinesterase activity is low, although its specific cells are in overwhelming preponderance per unit weight of tissues, it is clear that if these cells are the source of the enzyme, the concentration within the cells must be very low. However, the carotid body itself has very numerous nerve fibers within it (fig. 2), approximately as abundant as those within the adrenal medulla; it seems not impossible that such cholinesterase activity as does exist in the carotid body may be due largely to the presence of these sensory nerve fibers with their relatively low content of acetylcholine. This view is favored by the fact that the relative amounts of true cholinesterase and pseudo-cholinesterase are similar in sensory fibers and carotid body.

The experiments of von Euler and his co-workers indicate the release of a cholinergic substance during normal carotid body stimulation. True cholinesterase is of course a specific enzyme, and the methods used here in analysis would not, therefore, indicate the presence or absence of cholinergic substances other than acetylcholine. The experiments of Christie (1933), though interpreted at that time as proving an endocrine function for the carotid body, have indicated that carotid body tissue does contain a cholinergic substance; while similar in certain physiological properties this substance is not, according to Christie, acetylcholine. The present findings are in agreement with this latter conclusion; the low concentration of true cholinesterase found here does not support the assumption that the humoral element involved in carotid body stimulation is acetylcholine.

SUMMARY

The experiments of previous workers indicate that initiation of carotid body reflexes is normally dependent upon an alteration in pH of the chemoreceptor cells and a release of a cholinergic substance by these cells. A microchemical analysis of the carotid body has shown, however, that the true cholinesterase content of this organ is very low, in sharp contrast to the high true cholinesterase level characteristic of organs in which acetylcholine acts as a synaptic transmitter. The results of this analysis indicate, therefore, that the humoral mediator between chemoreceptor cell and sensory nerve fiber is not acetylcholine.

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OXYGEN CONSUMPTION OF EXCISED RAT TISSUES FOLLOWING ACUTE ANOXIC ANOXIA[1]

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Russell, Long and Wilhelmi (1944) recently reported a marked reduction of oxygen uptake in liver slices from rats in severe hemorrhagic shock, and considered that this was a consequence of lack of oxygen. Kidney slices from these bled rats showed little depression of respiration. Similar results were obtained after exposing slices of liver and kidney to an atmosphere of nitrogen *in vitro* (cf. Craig, 1943; Fuhrman and Crismon, 1944).

These results show that the liver is more severely damaged by anoxia than is the kidney. It is therefore of interest to examine other organs known to be sensitive to anoxia. In particular an examination of the heart and brain appears to be indicated. It is known that the mammalian heart can withstand anaerobic conditions for only very brief periods. Evidence of impaired mechanical response becomes apparent within one minute after clamping a coronary artery (Green and Wegria, 1942; Blumgart *et al.*, 1941). *In vitro*, rapid loss of the capacity for oxygen consumption follows semi-anaerobic incubation of rat heart at body temperature (Bernheim and Bernheim, 1944). In the brain, severe anoxia can lead to rapid development of a persistent electrical silence in the electroencephalogram (Kessler *et al.*, 1943). For comparison we have determined the rate of oxygen uptake of rat liver, kidney cortex, cerebral cortex, cardiac muscle and skeletal muscle after subjecting the animals to progressively decreasing atmospheric pressures until death occurred.

METHODS. Adult male albino rats of the Slonaker-Wistar strain weighing from 175 to 240 grams were used in the anoxia experiments. They were fed a stock diet consisting of dry dog food.

Six rats were made anoxic by placing them in small glass chamber which was evacuated by means of an aspirator. The pressure was measured with a mercury manometer and was regulated manually by means of a bleeder valve. Pressures of 440 and 320 mm. Hg were maintained for about 15 minutes. The pressure was then reduced 40 mm. each 10 minutes until respiration ceased. This occurred in all animals within 3 minutes after the pressure had been reduced to 80 mm. Hg. The animal was removed from the chamber 30 to 45 seconds after the last respiratory movement and transferred to a cold moist chamber (Fuhrman and Field, 1944) for preparation of the tissues. One rat was placed in a chamber containing soda lime and calcium chloride and permitted to rebreathe at constant atmospheric pressure until respiration ceased. Nitrogen was used to replace the oxygen as it was used.

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Tissue slices were prepared from cerebral cortex and heart ventricle by means of a razor blade and template (Fuhrman and Field, 1943), from kidney cortex with a modified Terry microtome (Terry, 1937) and from liver with a Martin (1942) slicer. Strips of skeletal muscle were teased from the adductor group of the hind leg (Hollinger, 1944). Oxygen consumption was measured by the direct method of Warburg in Ringer-phosphate solution containing 0.2 per cent glucose. The experimental details were the same as described elsewhere (Fuhrman and Field, 1943, 1944, 1945). Oxygen consumption is expressed in μl. per mgm. initial dry weight tissue per hour (QO_2). The QO_2 on an initial wet

TABLE 1

Oxygen consumption of excised tissues from normal rats and from rats subjected to acute anoxic anoxia

QO_2 determined in Ringer-phosphate with 0.2 per cent glucose at 37.7°C. Duplicate determinations were made on each tissue from a given animal. Rates of oxygen consumption remained constant for at least 60 minutes except in the case of heart where the QO_2 was calculated for the period during which it remained constant, usually the first 40 minutes after thermoequilibration.

TISSUE	MEAN WATER CONTENT* %	CONTROL SERIES			ANOXIC SERIES†		
		Number animals	Mean QO_2	S.E. of mean	Mean QO_2	S.E. of mean	P‡
1	2	3	4	5	6	7	8
Cardiac muscle	75.49	16	10.00	0.45	6.48	0.66	0.001
Liver§	69.42	7	9.83	0.17	7.87	0.15	0.001
Skeletal muscle	75.68	8	3.02	0.12	3.16	0.14	0.2
Cerebral cortex	80.59	9	10.64	0.19	10.99	0.26	0.3
Kidney cortex	75.66	9	16.52	0.71	16.81	0.36	0.7

* Water content for cerebral cortex and kidney cortex from Fuhrman and Field (1942), for liver from Fuhrman and Field (1945), for skeletal muscle from Hollinger (1944) and for cardiac muscle from unpublished results of this laboratory.

† The anoxic series consisted of seven animals.

‡ Probability that such differences in QO_2 as were observed between column 4 and column 6 would occur in random sampling.

§ Liver QO_2 corrected for glycogen content and expressed on a glycogen-free basis (QO_2GF) (Fuhrman and Field, 1945).

weight basis was converted to the QO_2 on an initial dry weight basis by correction for the water content determined on aliquots of each tissue (table 1, column 2).

Results. The results of the determination of oxygen consumption of normal tissues and tissues from rats dead from acute anoxia are given in table 1. Only in the heart and liver is the QO_2 of the tissue from the anoxic animals lower than that of the controls. In the case of liver the difference amounts to 20 per cent; for heart 35 per cent. The differences between the QO_2 of the control and anoxic skeletal muscle, kidney and cerebral cortex are not statistically significant. It is thus clear that anoxia of the degree and duration used results in damage of only the liver and cardiac muscle, if the criterion of damage be depression of oxygen uptake in glucose medium.

Discussion. It was to be expected that the respiration of excised liver would be depressed by anoxic anoxia in view of the findings of Russell *et al.* in which anoxia resulted from hemorrhage. Beecher and Craig (1943), however, found that oxygen uptake of the liver from hemorrhaged cats starved 24 hours was not significantly different from the controls. Russell *et al.* (1944) cite unpublished experiments showing a depressed oxygen consumption of liver tissue from cats in hemorrhagic shock after fasting 48 hours. Craig (1943) reported that anoxia *in vitro* depressed the oxygen consumption of liver from fasted rats more than that from fed animals. These differences are probably due to differences in glycogen content of the liver (cf. Fuhrman and Crismon, 1944). In the present experiments glycogen was determined on samples of fresh tissue from each animal. The mean glycogen content of the seven anoxic rats' livers was 0.79 per cent, a marked reduction below that of the controls (4.12 per cent). It is not certain that this reduction of liver glycogen *in vivo* during anoxia leads to a concomitant injury to the liver as a result of the reduced oxygen supply but there is reason to believe that this might be so. However, it seems probable that liver from rats with high glycogen reserves, as well as that from animals in which the liver glycogen is not so readily depleted by fasting (cats), may show less depression of oxygen consumption following anoxia.

Russell, Long and Wilhelmi (1944) reported that the different effects of glucose on the oxygen uptake of liver and kidney following anoxia "lend support to the interesting point that the order of resistance to damage of the tissues under these circumstances—liver < kidney < muscles—is the same as that of their ability to utilize glucose, both aerobically and anaerobically." The results reported here enable us tentatively to divide the tissues studied into two groups: 1, liver and cardiac muscle, characterized by relative inability to utilize glucose and low resistance to damage by anoxia; 2, cerebral cortex, skeletal muscle and kidney cortex, characterized by ability to utilize glucose and relative resistance to anoxia. Thus, van Harreveld and Tyler (1942) have shown that spinal cord regains, for a time at least, the initial level of oxygen consumption after as long as 60 minutes of asphyxia. When these tissues are subjected to an atmosphere of nitrogen *in vitro*, and the oxygen consumption subsequently determined in oxygen, the order of increasing resistance to damage by anoxia appears to be liver and cardiac muscle < kidney < cerebral cortex and skeletal muscle (unpublished data). Furthermore, it is of considerable interest that after acute high altitude anoxia in man one of the most consistent histological findings is the occurrence of fat-free, glycogen-free vacuoles in the myocardium and liver (Kritzler, 1945). These findings of the low resistance of cardiac muscle to acute anoxia are in harmony with those of Green and Wegria (1942) and others which indicate rapid loss of function following occlusion of the circulation in the intact animal.

SUMMARY

The rate of oxygen consumption of rat liver, kidney cortex, cardiac muscle, skeletal muscle and cerebral cortex was determined *in vitro* after subjecting the

animals to progressively decreasing atmospheric pressures until death occurred. When compared with the QO_2 of control tissues, the oxygen consumption of the liver and cardiac muscle of the anoxic rats was found to be decreased 20 per cent and 35 per cent respectively. The QO_2 of the remaining tissues studied was not significantly different from the controls.

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THE FAILURE OF TRANSFUSIONS IN IRREVERSIBLE HEMORRHAGIC SHOCK[1]

(A Study of Central Venous Pressures)

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It is generally appreciated by clinicians and experimentalists alike that tranfusions of ideal solutions are of only temporary benefit in advanced stages of shock. Well defined opinions exist as to why this happens. Probably the most generally accepted hypothesis is that cardiac output again decreases progressively because venous return fails, as it is known to do during development of shock. This only leads to another question, why is the returning volume of blood again reduced after transfusion? Two mechanisms are commonly invoked:

1. Transfused fluids, including their colloids, continue to be lost by virtue of the fact that capillary endothelium generally has become more permeable, as a result of prolonged vasoconstriction, hypotension, anoxia, toxemia, etc.

2. Venous blood becomes stagnant in capillaries and venules and cannot be moved onward, owing to loss of vascular tonus, diminution of surrounding tissue pressure, hypotonia of skeletal muscles, or failure of some other venopressor mechanism. The author has questioned whether substantial evidence exists that either capillary or venous mechanisms default significantly during shock (1). If either of these mechanisms is concerned with progressive failure of the circulation after substantial infusions of proper solutions have been administered, then it may be anticipated that central venous pressures should again fail.

In 1942 we (2) described a method for production of standardized shock which lends itself admirably to a comparative study of central venous—or right atrial—pressures after simple hemorrhages and during the progressive circulatory failure which follows several hours after reinfusion of the withdrawn blood.

Briefly, a dog anesthetized with morphine and sodium barbital is bled from a femoral artery at a rate of about 50 cc./min. until mean pressure is reduced to 50 mm. Hg. Such a pressure is maintained for 90 minutes by small additional withdrawals of blood. At the end of this period mean arterial pressure is reduced and maintained at a critical level of 30 mm. Hg for another 45 minutes by careful withdrawal of small additional amounts of blood. If a state of mild hyperthermia is maintained this practically always induces an irreversible state. The blood which has been withdrawn into heparin and kept cool is then warmed

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and reinfused at a rate somewhat less than 50 cc./min. Since the water lost by the dog through evaporation and urinary secretion is approximately corrected by occasional small infusions of saline solution, since the spleen remains constricted (3), and since fluid has entered the circulation from tissues (lowering of hematocrit readings), it may be assumed that the original circulating blood volume has been more than restored. However, the restoration of arterial pressure is not permanent; within ½ to 4 hours, cardiac output/min. (4) and arterial pressures decline progressively until the animal dies. It can be added that additional infusions of serum, artificial colloidal solutions, or saline are also ineffective. The condition obviously resembles one that occurs in man after large losses of blood, which is not benefited by adequate transfusions of blood, plasma or colloidal solutions.

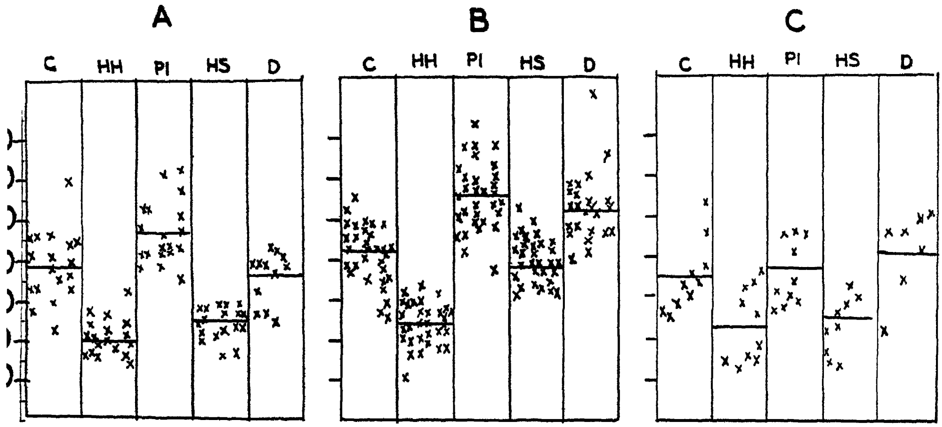

Fig. 1. Plots of three series of experiments showing effective venous pressures. *C*, control; *HH*, hemorrhagic hypotension; *PI*, post-infusion; *HS*, hemorrhagic shock following infusion; *D*, death. Discussion in text.

Effective venous (right atrial) pressures following loss of blood and during circulatory failure following reinfusion. Since right atrial pressure is affected, not only by the volume of blood returned and that removed per unit time by the heart, but also by changes of negative intrathoracic pressure, it is not permissible to base conclusions on venous pressures recorded by instruments balanced against atmospheric pressure. This is particularly true since we have found that intrathoracic pressure changes during the course of our experiments. Consequently, it is necessary to determine the respiratory changes of intrathoracic pressure simultaneously and to calculate the algebraic differences between these pressures and recorded venous pressures, i.e., the effective venous pressures. We have recorded such pressures by optical manometers during various stages of hemorrhage and post-transfusion failure and have calculated the mean effective pressure from such records during the brief phase of expiratory apnea.

The results of 48 such experiments are plotted as charts A and B in figure 1. Chart A shows results of 18 dogs which differed from a group of 30 dogs

in chart B. In both, columns *C* indicate control readings of effective mean atrial pressures and columns *HH* similar pressures 10 to 20 minutes after a stabilized 50 mm. Hg level of post-hemorrhagic hypotension had been reached. We notice in both sets of observations a general lowering of effective venous pressures during this period, the mean of all observations being indicated by a cross line. Following blood reinfusion after an additional 30 mm. Hg period of hypotension, effective atrial pressures usually rose to or above control values in individual experiments and the means are apparently higher in both groups of experiments (columns PI).

The effective mean venous pressures after arterial pressures had again declined to 50 mm. Hg—the intervals after infusion varying—are recorded in columns HS. In the group of 18 dogs plotted in chart A, effective atrial pressures were again reduced, but neither individually nor collectively to quite as low levels as during hemorrhagic hypotension. In the larger group of 30 dogs plotted in chart B, very little reduction occurred,—indeed, the lowest pressure was 60 mm. saline, a value commonly regarded as adequate for normal filling of the heart (Y. Henderson; Wiggers). The final column *D* indicates the effective pressures shortly after death in the animals in which it was reliably determinable. In the first group, these also were not low and, in the second, comparable to those on death of nonshock dogs. From such data it is difficult to draw the inferences, either that vascular capacity had increased significantly, or that the circulating blood volume had decreased. On the contrary, they strongly suggest that cardiac failure played a considerable part in the circulatory failure which occurred in the group of 30 dogs. However, before such a far reaching inference is drawn it is important to inquire whether some other interpretation can be made or whether some experimental error might have been introduced.

Actual atrial pressures after hemorrhage and restoration of blood volume. While calculations of effective atrial pressures constitute the only method for evaluating effective filling pressures, the question arose whether the usual methods for recording intrathoracic pressures can be subject to technical errors, particularly when employed over a long period of time. The natural pleural cavity consists of a minute layer of serous fluid between the two pleural layers. In order to measure pleural pressures the lung tissue is pushed away by means of a trocar, thus forming a small pocket. As a rule, successful recordings are not obtained unless a small amount of air is purposely or unwittingly introduced to maintain the pocket. Now the changes of pressure, recorded with equal expansion of the chest, depend on the size of air pockets, as can easily be verified by introducing volumes of air sufficient to constitute a partial pneumothorax.

If, during a long experiment, additional small quantities of air should surreptitiously enter, or if a part of the air should be absorbed, the size of the pocket would vary and the pressure recorded during inspiration and expiration would alter. Furthermore, it has never been demonstrated that the intrathoracic pressure is equal throughout the chest, nor is it probable that the negative pressures recorded from pleural or mediastinal spaces are quite equal to those which immediately surround the heart and large vessels. We therefore tried to eliminate

such a possible error by performing a series of 12 additional experiments in which atrial pressures were recorded optically after making an effective pneumothorax by retraction of a large incision in the third right intercostal space. Under such conditions, the thoracic vascular system and heart are under constant atmospheric pressure and the recorded right atrial pressure becomes the effective filling pressure for the right ventricle. Moreover, an exact constant placement of the atrial sound was assured through digital and visual examination. Our past experience was again duplicated. In spite of utmost precautions regarding anesthesia, operative trauma, hemostasis, etc., and with maintenance of arterial pressures, such operative procedures introduce some unknown factors in consequence of which animals withstand much less loss of blood. In some dogs, 15–20 cc./kilo sufficed to lower pressures to the 50 mm. Hg level, and the period, 30 mm. Hg hypotension, tolerated was much reduced. Furthermore, uncontrollable seepage of blood is apt to develop after reinfusion of heparinized blood. As a result, only three experiments were realized in which the bleeding volumes and periods of hypotension were comparable to those obtained in animals that breathe naturally.

All of the animals which tolerated less initial hemorrhage and hypotension showed about the same reduction of atrial pressure after transfusion as during the initial hemorrhagic hypotension, indicating that reduction of venous return was a significant factor. These results are shown in graph C of figure 1. It is impossible to determine the extent to which continued seepage of heparinized blood contributed to such reduction. The three animals that were more resistant to hemorrhage, but also developed shock after reinfusion, revealed mean atrial pressures which were slightly below or above those at the start.

Figure 2 shows segments of optical records from one of these experiments. Experimental data are given in the legend. Shortly after two successive initial hemorrhages of 220 and 195 cc. respectively (cf. B and C with A), and during persistence of the severe hypotension (D), the mean atrial pressure, determined by planimeter measurements, was definitely reduced. Mean atrial pressure was fully restored after transfusion (E) and increased still further during the next hour (F). During this time, arterial pressures and the form of the arterial pressure pulse did not deteriorate significantly. During the following hour (G), circulatory failure began and mean atrial pressure was more significantly reduced than the appearance of the curves indicates (see legend). During the next hour, however, venous pressures started to rise again (H) and this continued progressively until the end (I, J, K). Segment L shows a death pressure of 8.5 mm. Hg.

A study of the actual curves reproduced in figure 2 shows that mean atrial pressure is greatly affected by the amplitude of the atrial wave and the negative effect of each succeeding ventricular contraction. During the period of post-hemorrhagic hypotension (B-C, D), the atrial pressure is not only less during diastole but the atrial wave (A) is much smaller in comparison to that in segment A. However, the negative effect of ventricular systole also becomes less as the systolic discharge decreases. After transfusion we note the customary

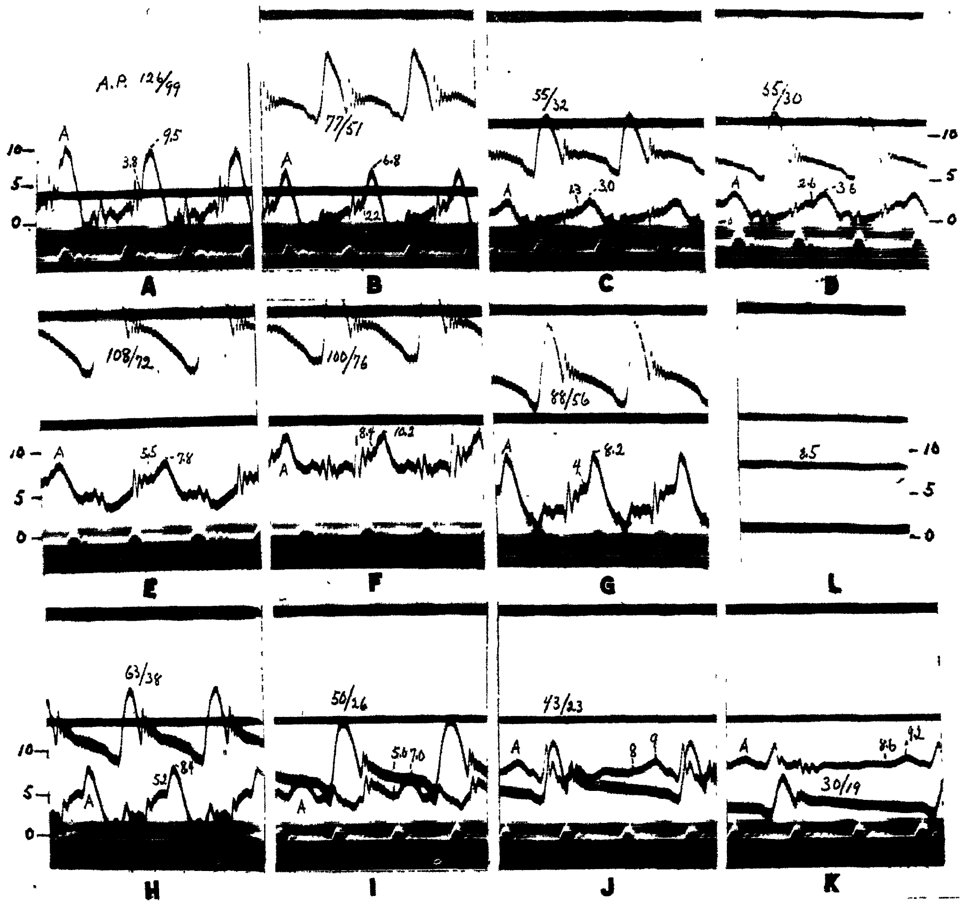

Fig. 2. Segments of records during posthemorrhagic hypotension, after infusion and during hemorrhagic shock. Systolic/diastolic pressures and venous pressures at beginning of inflow and summit of atrial contraction curve *A* marked on records. Note shift in atrial pressure in relation to base lines and recalibration between B and C and G and H. *L* = death pressure.

Calibrations in millimeters of mercury apply as follows: Upper left to A-B; upper right to C-D; middle left to E-F-G; middle right to L; lower left to H-I-J-K.

A—11.50—control 30 min. after opening chest—arterial pressure curves cut off to maintain uniformity of mounting—mean atrial pressure (MAP) = 4.5 mm. Hg; B—12.00—after 220 cc. hemorrhage, MAP = 3.0 mm. Hg; C—12.35—after total of 395 cc. hemorrhage, MAP = 2.3 mm. Hg; D—1.35—posthemorrhagic hypotension, MAP = 3.0 mm. Hg; E—2.25—4 min. after reinfusion of 325 cc. blood, MAP = 5.5 mm. Hg; F—2.35—same, MAP = 8.8 mm. Hg; G—3.30—beginning circulatory failure, MAP = 3.5 mm. Hg; H—4.30—same, MAP = 5 mm. Hg; I—5.10—same, MAP = 7 mm. Hg; J—5.15—same, MAP = 8.8 mm. Hg; K—5.16—same, MAP = 8.0 mm. Hg; L—death pressure, MAP = 8.5 mm. Hg.

slowing of the heart, a material elevation of diastolic atrial pressure, but no immediate restoration in the amplitude of the A wave, or the negative ventricular effect. It appears that, despite an increase in the initial tension, contraction

of the right atrium must be less forceful. This continues to be true in segment F. During the hour intervening between segments F and G, the diastolic atrial pressure fell progressively. This decline seems to be associated with a larger A wave and a greater negative ventricular effect. As circulatory failure progresses, these relations are reversed; diastolic atrial pressure rises and the force of atrial contraction diminishes progressively. Such curves raise the question whether ventricular filling and systolic discharge may not be determined more by the vigor of atrial contraction, as expressed by the summit of the A wave, than by mean atrial pressure.

The relation of atrial pressures to volume curves. It is one of the disadvantages of morphine-barbital anesthesia that the heart rate is usually increased far beyond the common range of tachycardia in man. Thus, in a series of 190 dogs, the

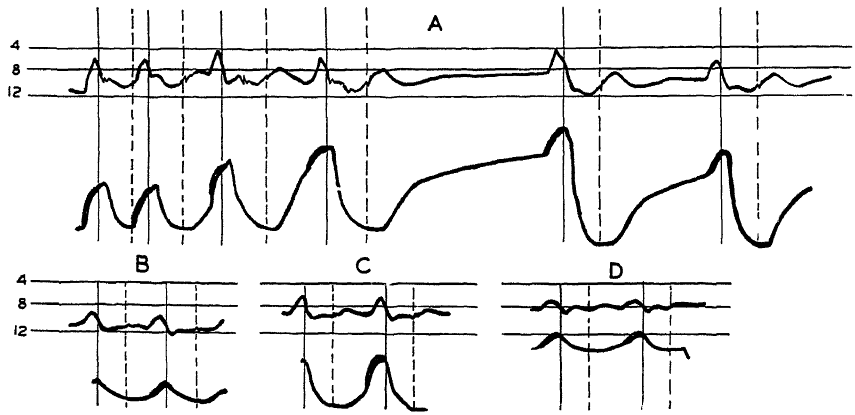

Fig. 3. Temporal relation of atrial venous wave (upper records) to ventricular filling as shown by volume curves of ventricles (lower curves). *A*, control at different cycle lengths; *B*, during hemorrhagic hypotension; *C*, after reinfusion of blood; *D*, during subsequent hemorrhagic shock.

rate ranged from 155 to 185 in 55 per cent, from 185–212/min. in 11 per cent, and averaged 97 in only 34 per cent. Significant operative procedures usually increase heart rate above 155/min. in all animals. This has the effect of bringing the whole period of ventricular filling during atrial systole. The altered relationship of atrial systole to ventricular filling as the heart slows is shown by the series of transcribed curves of figure 3-A, in which progressive slowing followed vagus stimulation. We note that at the initial heart rate of 230/min. ventricular filling occurs entirely during atrial systole, but, when the heart slows progressively, increasing periods of rapid inflow and disastasis precede atrial contraction. During the 50 mm. Hg period of hypotension (B) when atrial pressures are reduced, sufficient slowing occurs to allow a short interval of inflow previous to atrial contraction. After reinfusion of blood (C) the heart has slowed a little more and, owing to elevated venous pressure at the moment the A-V valves open, a larger hydrostatic inflow is made possible, but the dominant filling still occurs during atrial systole. As circulatory failure develops and with

essentially the same heart rate, ventricular filling is reduced as a result of both decreased hydrostatic effect and weakened atrial systoles. Such analyses indicate that, while reduction in the force of atrial systole contributes to reduced ventricular filling, it is not the only factor involved; essentially similar effects would operate were the heart rate slower. The fact that the ventricles are extremely dilated at the time indicates that myocardial depression rather than impaired filling pressure is the cause of the reduced systolic discharge.

Static equilibrium during hemorrhage and shock. In 1935 Y. Henderson and associates (5) recalled, repeated, and reinterpreted an experiment reported by Riml in 1928: The pulmonary artery of a cat is suddenly clamped and the pressure and volume of blood flow from a trocar inserted into the right atrium are determined. A half or more of all the animal's blood (3-3.5 per cent body weight) runs out in three minutes under an initial pressure to 10 to 12 cm. of blood. This propulsion was attributed by Y. Henderson et al. (5) to a force exerted by tissue pressure which, in turn, was attributed to muscle tonus. The fact that most of this blood does not come from muscles was ignored.

The experiment is not essentially different from one in which heart action is abruptly stopped and has generally been explained by establishment of hydrostatic equilibrium throughout the vascular system, as a result of which the larger part of the circulating blood accumulates in the more capacious veins and chambers of the right heart. It is also a corollary of the static equilibrium concept that the volume and pressure of blood in the veins and the right heart should rise and fall with increase or decrease in circulating volume.

For this reason we measured the effective venous pressures immediately after death. As already analyzed, in the majority of shock animals the death pressures were not significantly different from those found in animals that do not die of shock. However, since death pressures can be measured only once and comparisons must be made between different groups of animals, we attempted to study pressures during static equilibrium a number of times in the same animal. This was done by the simple expedient of producing complete cardiac inhibition by stimulating the peripheral end of a vagus nerve. This inhibition was sustained sufficiently long in six experiments on open chest animals to lower arterial pressures to 28 or 30 mm. Hg. A typical result of such an experiment is shown in figure 4. Graph A shows data regarding the decline of arterial and the rise of right atrial pressures under control conditions. As expected, the latter rose progressively to a fairly stable level. Graph B plotted from records during the period of 30 mm. Hg hypotension shows no rise of venous pressure. Graph C, giving data during vagal inhibition about 10 minutes after reinfusion, shows that, while venous pressures start a little below control A, a progressive rise to practically the same end point as in curve A occurs. (Note different ordinate scales for curves A,B and C,D.) Graph D gives data from another test made after post-infusion circulatory failure had progressed to a 50 mm. Hg level of arterial pressure. In comparison with curve B a significant rise of venous pressure resulted. Unfortunately, the final stimulation led to death of the animal with venous pressures essentially those produced initially by vagal stimulation.

Such results again fail to support the "loss of tonus" theory if Henderson's postulates are accepted, or any "exemia" theory of shock if the common explanation of static pressure equalization holds.

DISCUSSION. During the past three years we have developed and improved (1) a method for production of standardized hemorrhagic shock. In addition, we have studied fairly completely the incidence of hemodynamic changes during the two periods of posthemorrhagic hypotension and during the circulatory

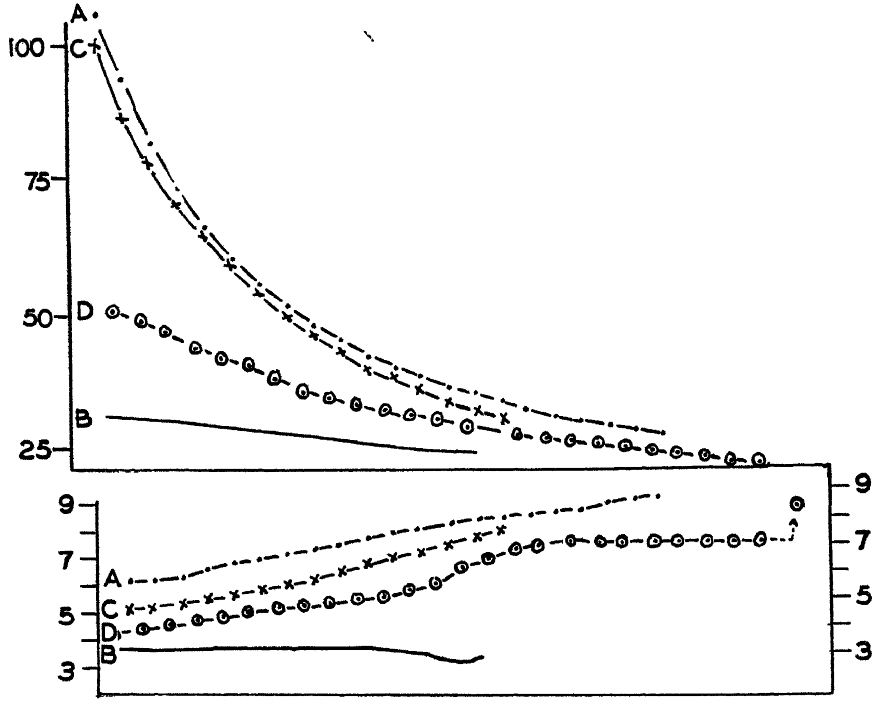

Fig. 4. Plots showing decline of arterial pressure (upper series) and changes in central venous pressures (lower series) during prolonged vagal inhibition of heart.

A, control; *B*, during 30 mm. Hg period hypotension; *C*, 5 min. after completion of reinfusion; *D*, during development of hemorrhagic shock; mean arterial pressure 58 mm. Hg; ordinates for arterial pressure curves in millimeters of mercury; for venous pressure curves in millimeters of mercury. To prevent overlap, venous curves *C* and *D* are plotted according to slightly raised scale on right.

failure which follows reinfusion. The trend of changes is brought together in figure 5. Briefly, the hematocrit readings decreased progressively during the periods of moderate and marked posthemorrhagic hypotension, but gradually returned toward or to the normal after reinfusion of blood. Cardiac output per minute and systolic discharge, determined both by cardiometric (6) and by a modified Stewart method (4), were reduced to 29 to 45 per cent of control values during the posthemorrhagic hypotension periods. After reinfusion, systolic discharge was often increased greatly, but, owing to persistence of a

slower heart rate, the minute output of the ventricles rarely quite reached control values (average ca 85 per cent). During the subsequent period of circulatory failure, cardiac output/min. dropped progressively. Changes in total peripheral resistance calculated from measurements of cardiac output/min. and mean arterial pressures were extremely variable, sometimes increasing and at other times decreasing during the posthemorrhagic hypotension period and early periods following reinfusion (4). However, as arterial pressures progressively failed, total peripheral resistance rather consistently decreased. The spleen contracted sharply during the first hemorrhage and generally remained so until

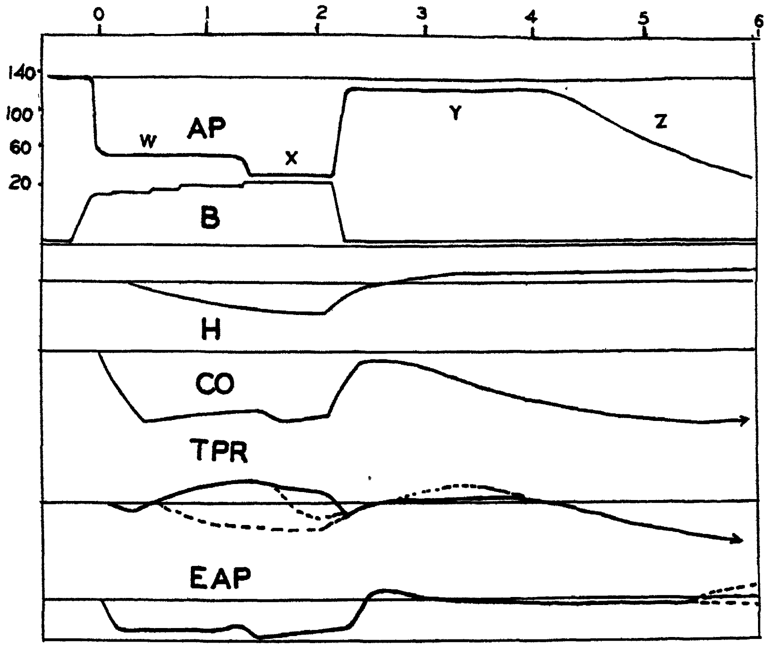

Fig. 5. Summary of average course of events during moderate hemorrhagic hypotension, *w;* severe hypotension, *x;* after reinfusion, *y*, and hemorrhagic shock, *z*. *B*, bleeding and reinfusion; *H*, hematocrit trend; *CO*, cardiac output/min.; *TPR*, total peripheral resistance; *EAP*, effective atrial pressure (dominant trend).

the end (3). In short, the general course of events corresponded to that generally accepted in various types of shock. However, the generally accepted corollary that the reduction of cardiac output per minute after transfusion is due solely to progressive reduction of venous return is not supported by data in this and previous communications.

Without question, when circulating volume is appreciably reduced, as in large hemorrhages or through great loss of plasma, arterial pressures decline progressively to low levels, chiefly because an insufficient volume of blood is returned to the heart (*initiating factor*). Our stage of drastic hemorrhagic hypotension is comparable to such conditions. If such a state of hypotension

is severe and prolonged enough, it leads to death from respiratory or myocardial failure (*lethal factors*). Slowing of the heart, slight rise of central venous pressure, decreased force of atrial contraction, and slight increase in cardiac size (7) are earmarks of such terminal cardiac failure.

If death does not supervene, irretrievable damage of an unknown nature generally—but not always—occurs; a state of hemorrhagic shock develops. Unfortunately, the existence of such an irreversible state cannot be detected otherwise than by the sustained response of the heart and blood pressure to reinfusion of the withdrawn blood. For laboratory studies we have therefore not declared an animal in shock until failure of such a sustained response had been demonstrated. Furthermore, since the animal develops circulatory failure despite restoration of the normal blood volume in the body, we felt that conditions might be comparable to the type of shock in which hemorrhage or loss of plasma volumes appear not to be initiating factors. Such correspondence remains to be demonstrated.

For the present, our results are deemed valuable because 1, they permit some estimate of the nature of the irretrievable damage which follows loss of large fractions of blood or plasma, and 2, they shed light on the problem of why transfusions may prove of only temporary benefit in advanced stages of shock initiated by decrease in circulating volume. Careful postmortems of dogs presented in this and previous communications indicate that some pooling of blood and transudation occurs, but this is limited largely to the intestinal mucosa and even here varies a great deal in intensity. It is conceivable that in dogs in which engorgement, edema, and transudation extend far down the jejunum and ileum, sufficient fluid might be lost from the circulation to reduce venous return. This was apparently the case in the group of dogs charted in figure 1-A, in which effective venous pressures were rather low during hemorrhagic shock following transfusion and after death, for a review of protocols shows that the gut changes were marked 3+ or 4+.

However, in many experiments the mucosal changes were rather mild and limited definitely to the duodenum (1+ or 2+). Since the spleen always remained firmly contracted and the hematocrit readings never exceeded controls very much, it becomes difficult to visualize how, in these cases, enough fluid could have been abstracted from or sequestered in the vascular system to decrease circulating volume to the point that led to circulatory failure and death. Our researches on hemorrhagic shock strongly suggest that in these cases persistent low pressure damages the myocardium more than peripheral vessels and that this is the major factor in the failure of blood transfusions in such advanced stages of shock. The possibility has also not been ruled out that such myocardial depression remains a hidden factor in those cases in which reduced effective venous pressures obtain. Our reasons for such a belief are as follows:

1. H. C. Wiggers and Middleton (4) found that cardiac output/min. is rarely restored fully after transfusion (average 85 per cent) and that it quickly decreases after transfusion has been completed. Unfortunately, the method employed in these determinations prevented simultaneous studies of effective venous pres-

sures. *2*. The results of very similar experiments, considered individually or as means, which are presented in this communication show that effective right atrial pressures are somewhat above control values after reinfusion. The conclusion seems obvious from these complementary investigations that the myocardium is unable to respond with equal stroke volumes to identical or slightly raised filling pressures immediately after transfusion. *3*. While the investigations of H. C. Wiggers and Middleton (4) have shown that a progressive reduction of cardiac output accompanies the circulatory failure following transfusion, results reported in this paper reveal that in the majority of experiments this is not accompanied by significant reduction in central venous pressures, and that the hydrostatic rise of venous pressure following cardiac standstill is essentially normal during such circulatory failure. This is difficult to interpret otherwise than that perhaps a reduced, but still adequate, volume of blood remains in circulation and that peripheral mechanisms permit an adequate return to the heart under hydrostatic forces.

SUMMARY

If reduction in circulating volume and in venous return, due either to exemia or to failure of a venopressor mechanism, are dominant factors in the circulatory failure which follows substantial infusions of adequate solutions, it may be anticipated that effective central venous pressures are reduced, as they are after large hemorrhages. We found this true in only 18 out of 48 dogs submitted to our standardized technique for production of hemorrhagic shock.

The additional observations that effective venous pressures immediately after death from hemorrhagic shock are essentially within normal ranges, and that the static circulatory equilibrium produced by prolonged vagal inhibition of the heart is also essentially normal, fail to support the views that capillary transudation, pooling of blood in the vascular system, or failure of any venopressor mechanism represent the essential irreversibility factor in the majority of our animals.

The results strongly suggest that failure of transfusions in shock are not necessarily due to unsuitability of solutions used nor chiefly to loss of plasma or solutions through damaged capillaries or failure of a venopressor force, but, frequently at least, are attributable to myocardial depression, i.e., to a reduced reaction to equivalent venous filling pressures.

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BLOOD POTASSIUM AND HISTAMINE INTOXICATION IN RELATION TO ADRENOCORTICAL FUNCTION IN RATS[1]

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The possible explanations for the several fold increase in susceptibility to histamine in the adrenalectomized animal, such as decreased histaminase activity or general increased capillary permeability, have not been entirely satisfactory (1, 2). In 1937 Zwemer and Truszkowski (3) suggested that the enhanced sensitivity might be related to the elevated blood potassium following histamine injection, as noted by Kuschinsky (4) and Thaler (5). Although the theory that various types of shock and adrenal insufficiency are the result of the toxic effects of potassium is not entirely supported by subsequent evidence (*cf.* 6), the situation with regard to histamine intoxication has not been specifically investigated so far as we are aware. Perla was able to raise the resistance to histamine in normal rats by the administration of saline solution (7), and Perla and Sandburg (8) decreased it by producing an electrolyte disturbance analogous to that following adrenalectomy by intraperitoneal injection of isotonic glucose solution. In some preliminary experiments in a small series of rats we attempted to test Zwemer and Truszkowski's proposal concerning the effect of elevated blood K on the reaction to histamine. Since one cannot rely upon single or repeated intraperitoneal injections of the maximum tolerated amounts of potassium to effect a sustained rise in K level, the procedure seemed inconclusive, but large amounts of potassium given prior to the injection of histamine, 50 mgm./100 grams body weight, did not modify its toxicity. Further observations, therefore, on the toxicity of potassium in relation to adrenocortical function, and the blood K after histamine, were made and are reported below.

Rats with autoplastic adrenocortical transplants were eminently suitable for this work because their response to potassium was like that of the normal rat although they were more susceptible to it, yet their susceptibility and reaction to histamine were more like those of the adrenalectomized animal. Also, the rat with transplants has sufficient adrenocortical protection to maintain growth and circulatory standards under optimum basal conditions, but not under stress, so its initial condition is not as equivocal as that in the totally adrenalectomized rat. Moreover, the technical difficulties in blood studies promised to be less.

METHODS. The survival experiments after intraperitoneal injection of 10 per cent potassium chloride or acetate were done on young adult males and females of an inbred strain of albino rats. Adrenalectomized rats at least two weeks after operation and rats with autoplastic dorsal intramuscular transplants of

[1] This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

adrenal cortex, two to three months after operation were used. Serum K was determined on intact male rats and male rats with transplants of the Long-Evans strain, using a colorimetric modification by one of us (M. A. D.) of the Consolazio and Talbott chlor-platinate method for small samples of serum (9). Blood was obtained under urethane or amytal anesthesia from the vena cava or aorta of untreated or histamine (ergamine acid phosphate, Burroughs, Wellcome & Co.) treated rats. Whole blood samples from the tail were analysed for total K; cell volume and hemoglobin were determined by the photocolorimetric methods of Shohl, Blackfan and Diamond (10).

RESULTS. *Toxicity of intraperitoneal injections of K.* The maximum tolerated amount of K was approximately 25 mgm. (as K+)/100 grams body weight for the normal rat (table 1). This agrees with the findings of Silvette, Britton and Kline (11). This dosage killed 69.6 per cent of the rats with transplants but only 6.5 per cent of the normal rats. One-half or less of this amount was fatal to all adrenalectomized rats. Our figures for normal and adrenalectomized animals compare favorably with those given by Rapoport and Guest (12). With

TABLE 1

Toxicity of intraperitoneal injections of K

DOSAGE OF K^+	NORMAL RATS			RATS WITH TRANSPLANTS			ADRENALECTOMIZED RATS		
	Number injected	Died	%	Number injected	Died	%	Number injected	Died	%
mgm./100 grams body weight									
10–17	27	0	0	26	0	0	13	13	100
25	46	3	6.5	23	16	69.6			
37	31	31	100						

smaller dosages all the rats with transplants recovered fully without developing signs of secondary shock or the prospect of delayed death, in contrast to adrenalectomized rats. The blood sugar of both rats with transplants (6 cases determined) and normal rats (3 cases) rose to 146–174 mgm./100 ml., indicating sympathetic activity, without the mediation of adrenin in the former. In adrenalectomized rats (5 cases) the blood sugar fell to 69-80 mgm./100 ml. during the first hour, and to 41–59 mgm. at death in the third hour.

The symptoms of potassium poisoning in the adrenalectomized rats differed from those in rats with intact glands or transplants of adrenocortical tissue. The former gradually developed a shock-like condition with hypoglycemia. In the latter, after fatal amounts of K, acute asphyxial convulsions and death with hyperglycemia occurred in less than an hour, and moist hemorrhagic lungs and extreme flaccidity of the heart were found at autopsy.

K levels in unoperated male rats. In tail blood specimens the total K averaged 246 mgm./100 ml. in 19 rats (table 4). Rapid and temporary rises to over 300 mgm./100 ml. were frequent. The serum K in venous blood averaged 21.3 mgm./100 ml. in 10 cases (table 3) and in aortic blood 21.1 mgm./100 ml.

in 6 cases. Single or repeated bleedings totaling more than 6 per cent of the blood volume (3 to 4 ml.) produced a varying degree of unavoidable hemolysis, which in the normal rat did not exceed an amount accounting for more than 2 to 3 mgm. of K/100 ml., i.e., a rise of 10 to 12 per cent (table 2). In addition there seemed to be a small increment independent of hemolysis, possibly the result of sympathetic stimulation.

After intraperitoneal injections of 5 and of 10 mgm. histamine per 100 grams body weight in the normal rat total blood K had risen an average of 7 and 11 per cent of the initial values by the end of the first half hour (table 4). These increments are of questionable significance since a normal variation of 10 per cent may be met in determinations on the small blood samples employed (0.05 and 0.1 ml.). Serum K also rose (table 3), but again the rises were probably not significant since in order to establish conditions comparable with those where hemoconcentration made the maximal possible bleeding only just sufficient for a serum K determination (e.g., in rats with transplants after histamine) the rats were bled as completely as possible in all cases and the factor of hemolysis must

TABLE 2

Effect of bleeding on venous serum K of untreated normal rats and rats with transplants

AMOUNT OF BLEEDING	NORMAL RATS		RATS WITH TRANSPLANTS	
	Number of rats	Serum K	Number of rats	Serum K
		mgm./100 ml.		*mgm./100 ml.*
First bleeding *ca* 4 ml.	10	21.3 ±1.3*	10	25.6 ±2.7
Second bleeding *ca* 4 ml.	5	23.8 ±1.4	8	30.6 ±3.5
Single maximal bleeding *ca* 7 ml.	10	23.8 ±2.2	11	27.8 ±2.4

* Standard deviation.

have operated to raise the serum K somewhat. Nevertheless the consistency of the findings on the high side of the normal range makes us feel that a very small rise in K actually occurred after histamine, although a more extensive series and use of larger amounts of histamine would be required to verify it. In any case our data do not vitiate previous findings in species other than the rat.

At the end of one hour the total K and serum K had returned to approximately the initial levels. Changes in cell volume and hemoglobin were also followed (see table 4).

K levels in rats with adrenocortical transplants. In the rats with transplants hemolysed sera occurred more frequently than in the normals, and in the histamine treated cases more than half of the samples had to be discarded for this reason. Clarke and Cleghorn (13) suggested that in rats intravascular hemolysis occurs in shock. This may have been true of our severe cases, but ordinarily the difficulty in avoiding hemolysis was correlated with sluggishness of flow, paralleling the degree of hemoconcentration. Also, the fact that 0.02 ml. of unhemolysed blood could be obtained from the tail during stasis shows that it is not a usual event in vivo. Moreover, if intravascular hemolysis had occurred

the increase in cell volume would have been consistently less than the rise in Hb determined on the same sample of blood.

The serum K in the first 4 ml. specimens of vena caval blood averaged 25.6 mgm./100 ml. (table 2). Allowing for traces of hemolysis and individual variation which is greater than in the unoperated rat it is concluded that the K level in the rat with transplants may run about 10 per cent higher than in the normal rat. The serum K of single 6 to 7 ml. specimens was 27.8 mgm./100 ml. and that of second 3 to 4 ml. samples was 30.6. Although the amount of hemolysis was slightly greater than in the normal rat it does not account for all of the increase. The total blood K from the peripheral circulation, as compared with serum K, showed less fluctuation and averaged 227 mgm./100 ml. (table 4).

After 5 mgm. of histamine/100 grams body weight the symptoms of shock in the rats with transplants were more pronounced than in the normal rat, but by

TABLE 3

Effect of intraperitoneal injection of histamine on venous serum K of normal rats and rats with transplants

HISTAMINE	MINUTES AFTER INJECTION	NORMAL RATS		RATS WITH TRANSPLANTS	
		Number of rats	Serum K	Number of rats	Serum K
mgm./100 gram body weight			*mgm./100 ml.*		*mgm./100 ml.*
5	30	3	24.7 ±0.2	3	35.3 ±4.2
	60	5	22.2 ±1.4	3	34.5 ±4.2
10	30	6	24.5 ±1.7	6	34.2 ±5.8
	60	8	22.9 ±3.5	2*	38.5 ±6.5
				3†	56.1 ±3.8

* Recovering.

† Dying.

the end of an hour some improvement in their condition could be detected. In half an hour the total K had risen 38.6 per cent and was as high or still higher at the end of an hour (table 4). At this time the serum K was also elevated, around 35 per cent (table 3). Because of the sluggish circulation it was difficult to obtain sufficient blood for serum K determinations and the values obtained were all affected to some extent by hemolysis. In one case, however, which recovered better than the others, unhemolysed serum was obtained and the K level was 30.2 mgm./100 ml., which agrees with the figure for the group after application of the calculated correction for hemolysis (4–5 mgm./100 ml.). Cell volume and hemoglobin increased in the first half hour and were at about the same levels at the end of an hour (table 4).

Within an hour after 10 mgm. of histamine/100 grams body weight about two-thirds of the group were dead or dying, so reliable samples of venous blood were obtainable in only two cases which were recovering at the end of an hour. In one with no hemolysis serum K was 32 mgm./100 ml. and in the other with moderate hemolysis 45 mgm./100 ml. (table 3). In three other rats with transplants in profound shock the K after correction for the hemolysis found in the

sera was 51–60 mgm./100 ml. The rise in total K was accompanied by marked hemoconcentration but not enough to account for all of the K increase (table 4). In an hour the total K, cell volume, and Hb all showed a further increase.

DISCUSSION. Small amounts of histamine tend to increase the serum potassium and the total potassium very slightly if at all in the normal rat, while in the rat with transplants these same amounts cause a rise which is four or five times as great and lasts twice as long. The elevation in total K roughly follows the changes in hemoconcentration, especially in the latter. An hour after a 5 mgm. dose of histamine per 100 grams body weight the percentage increases of the total K, Hb and cell volume in the normal rat showed approximately a 1:1:1

TABLE 4

Changes in the peripheral blood of normal rats and rats with transplants after intraperitoneal injection of histamine

HISTAMINE	MIN. AFTER INJECTION	TOTAL K			CELL VOLUME			HEMOGLOBIN		
		Number of rats	Mgm./100 ml.	Per cent increase	Number of rats	Ml./100 ml. blood	Per cent increase	Number of rats	G./100 ml. blood	Per cent increase
					Normal rats					
mgm./100 g body weight										
0	0	19	246		19	44.4		22	13.8	
5	30	8	264	7	8	49.9	13.4	7	15.1	9.6
5	60	10	252	2.2	8	45.1	2.2	8	14.1	2.4
10	30	3	274	11.1	4	54.6	23.7	5	16.2	17.5
10	60	5	257	4.5	5	50.4	13.9	4	15.8	14.5
					Rats with transplants					
0	0	19	227		22	42.5		19	13.9	
5	30	5	315	38.6	7	56.6	33.1	7	17.7	26.7
5	60	9	319	40.8	9	55.3	30.1	6	18.0	28.9
10	30	6	363	59.7	8	60.5	42.4	8	19.2	37.1
10	60	2	370	63.0	3	66.3	56.2	3	21.8	56.2

relationship and were not significantly above the resting values, whereas in certain cases among the rats with transplants the potassium remained high as the cell volume and/or the Hb began to decline. This together with the persistent elevation of serum K indicates that in the rat with adrenocortical tissue only, after histamine blood potassium is maintained at a high level by transfer of extravascular potassium as well as by hemoconcentration.

The actual levels of serum potassium found in rats with adrenocortical transplants after histamine injection are not considered to be toxic *per se* by most authorities (14, 15), but as Clarke and Cleghorn (13) point out, such concentrations of potassium along with hemoconcentration may have an entirely different effect on the organism than a simple rise without other complications. Since the initial serum potassium tends to be higher than in the untreated normal rat, the rise after histamine greater and more enduring, and the susceptibility to potassium increased, it would appear that potassium should be considered as an

important factor in histamine intoxication. Death from potassium poisoning differs from histamine shock in the rat with transplants, however, in that in the former acute cardiac failure predominates over peripheral circulatory failure. It is possible that in histamine shock enhanced levels of potassium develop so slowly, as compared with the intraperitoneal administration of the cation, that they merely aggravate the circulatory disturbances already instituted. The enhanced toxicity of histamine in the rat with transplants would seem, therefore, to be fundamentally correlated with a disability in the homeostasis of body fluids, as we have noted before in connection with massive glucose injections (16). The resulting elevation of potassium would be only a contributory factor, especially effective, however, since these animals are more susceptible to K. Thus the intermediacy of the rat with transplants between the normal and the totally adrenalectomized animal with regard to functional efficiency of adrenocortex is further emphasized.

SUMMARY

1. About 70 per cent of rats with adrenocortical transplants were killed by doses of potassium salts (25 mgm. K^+/100 grams body weight) which were well tolerated by normal rats, but were at least twice the size of doses invariably fatal for adrenalectomized rats.

2. After intraperitoneal injections of histamine (5 or 10 mgm./100 grams body weight) the total blood K and serum K of normal rats may have risen slightly although the observed changes were within the range of normal and experimental variation. In the rats with transplants there were increases in serum K and in total K which were greater than could be accounted for by the accompanying hemoconcentration.

3. It is suggested that although the increased susceptibility of the rat with transplants to histamine is fundamentally related to a disturbance in handling body fluids because of some degree of functional inefficiency of the grafted cortical tissue, the elevated potassium may be a contributory factor which aggravates the circulatory disturbances.

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PROTECTION OF ADRENALECTOMIZED RATS AGAINST A HIGH TEMPERATURE

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Patients suffering from adrenal insufficiency appear to be more susceptible to hot weather than are normal individuals. The importance of the adrenal in susceptibility to heat is shown also under certain conditions in animals that have been deprived of these glands. In a short paper some years ago it was shown that adrenal cortical extract furnished a considerable measure of protection for adrenalectomized rats exposed to heat (6). In the present investigation an attempt has been made to compare the various treatments, now in vogue for adrenal insufficiency, in their ability to enable the adrenalectomized rat to withstand exposure to heat.

METHODS. The animals were maintained in an atmosphere of approximately uniform temperature by means of a large, insulated water bath, the temperature of which was thermostatically controlled. Each rat was placed in a one liter beaker containing a 1 kgm. weight resting on absorbent cotton. A screen of half inch mesh was used as a cover. Twelve beakers, submerged for two-thirds of their height in water, were supported by a shelf located in the upper level of the bath. Circulation of the water was maintained by a motor driven screw. The bath had a partial cover (5 in. above the top of the beakers) which prevented major air currents from entering but allowed adequate ventilation. The relative humidity within the beakers containing the rats was from 90 to 100 per cent during an experiment.

Colonic temperatures were taken immediately before, at hourly intervals during and one hour after the end of the test. A clinical thermometer was inserted into and beyond the evacuated rectum for the same distance each time. Each rat was out of the bath for about three minutes every hour for this purpose. Body weights were taken at the time of adrenalectomy and just before and just after heat exposure.

Bilateral adrenalectomy, under ether anesthesia, was performed at one operation through a single skin incision and through lumbar approaches. Following the operation the animals were kept at a temperature of 21 to 24°C. Purina dog chow and water were available at all times.

Something must be said about the size or age of the animal which is necessary for this study. Rats weighing 95 to 150 grams appeared to show a greater resistance to heat than did larger, older animals. This in itself would be no objection, but it seemed impossible to find a temperature which would permit survival of most of the normal animals and still cause most of the untreated adrenalectomized animals to succumb. On the other hand, most normal rats ranging from 200 to 340 grams in weight survived at 38.7°C. (101.7°F.) while

most of the adrenalectomized rats of this size died. Therefore, this temperature and range in body weight were used throughout these experiments. Tests usually began at 9 a.m. and continued for a period of four hours. A few normal rats were always exposed with the adrenalectomized animals. Rats were tested three or four days after adrenalectomy since the results were less uniform on the first and second days.

A total of 453 rats, 438 males and 15 females, were used for the final study. Seventy were used as controls, being exposed to the same conditions but not operated. Of the adrenalectomized animals, 76 were untreated; 75 were given one per cent sodium chloride solution to drink *ad lib*; 101 were treated with desoxycorticosterone acetate (DCA)[1]; 35 were given adrenal extract.[1] Ninety-eight of these, both normal and operated animals, were used for the study of tissue water.

The DCA in oil (1 cc. = 5 mgm.) was given to the adrenalectomized animals subcutaneously in one milligram doses beginning the day of operation. At first, 1 mgm. doses were given daily up to the day of exposure. Thus a total of 3 and 4 mgm. were given to the three and four-day animals respectively. It was found that this dosage might be cut to 2 and 3 mgm. Under this regimen 1 mgm. was given on the day of operation, and for one or two days following, but none the day before or on the day of the test.

Those adrenalectomized rats receiving adrenal extract were injected subcutaneously with two 0.5 cc. doses daily until the morning of the experiment. Just before exposure to heat each animal was injected with 0.5 cc. subcutaneously and 0.3 cc. intraperitoneally. In a few animals only 0.4 cc. and 0.3 cc. were given for the last two doses.

The animals used for tissue studies were selected at random from the different groups. After being anesthetized with nembutal, a piece of skin approximately one inch square was removed from a previously shaved and dry abdomen. Blood (0.5 cc.) was taken from the heart. A 0.2 to 0.4 gram sample of gastrocnemius muscle was removed and the right cerebral hemisphere, freed from meninges and large blood vessels, was saved.

The four different tissues were weighed immediately after removal and then dried in an oven at 102°C. for three days. After being placed in a desiccator for a fourth day they were weighed and checked to constant weight.

RESULTS. Upon exposure to heat all animals were very active but soon stretched themselves out as much as space allowed. The control rats and adrenalectomized rats treated with adrenal extract or with DCA remained restless and excitable. The untreated and sodium chloride-treated adrenalectomized animals became quiet and usually unresponsive. However in the late stage they were occasionally thrown into convulsions by handling. Respiration became rapid and shallow. Excessive salivation occurred in all of the animals, and many of them frantically washed their faces. In the case of the normals this salivation

[1] We are indebted to Dr. R. D. Shaner of Roche-Organon Inc., Nutley, N. J., for desoxycorticosterone acetate and to Dr. G. F. Cartland of the Upjohn Company, Kalamazoo, Mich., for adrenal extract.

was copious, so that by the end of four hours they appeared as though they had been dipped in water. Exophthalmia was a constant finding until near death. Spasmodic blinking of the eyes was observed in some as they reached their temperature peaks. A frank respiratory distress with violent gasping was also observed. This was most evident in the untreated animals.

Cyanosis of ears, testes and, particularly, the extremities was observed in all of the animals when they were removed from the heat. At autopsy, in all of the animals that died during exposure and in those used for tissue work, the internal organs showed marked vascular engorgement.

Survival. The survival of adrenalectomized rats treated by various methods is shown in table 1. Ninety-three per cent of the normal rats survived while only 14 to 17 per cent of the untreated adrenalectomized rats survived. Sodium chloride afforded some protection since 53 to 27 per cent of the adrenalectomized rats that were permitted to drink 1 per cent NaCl survived. The former were

TABLE 1

Survival of rats exposed to 38.7°C. for 4 hours

CONDITION	NUMBER OF ANIMALS	SURVIVAL IN PERCENTAGE
Normal	70	93
3 days after adrenalectomy (no treatment)	41	17
1% NaCl-treated	36	53
DCA-treated	64	78
Adrenal extract-treated	35	89
4 days after adrenalectomy (no treatment)	35	14
1% NaCl-treated	37	27
DCA-treated	37	89

tested three days after adrenalectomy while the latter were exposed four days after adrenalectomy. DCA treatment was nearly adequate since 78 and 89 per cent survived. About the same survival was obtained by treatment with adrenal extract, namely, 89 per cent.

The reaction of the animals as indicated by their body temperatures is shown in table 2 wherein the temperatures of the rats that survived have been averaged. All but the DCA-treated adrenalectomized rats had a lower temperature at the start than did the normal rats. In all animals the greatest rise occurred during the first hour, and then increased more slowly to a peak which was reached in the third hour in the normal, DCA-treated adrenalectomized and extract-treated adrenalectomized rats and in the fourth hour for the others. One hour after removal from the bath the DCA-treated rats showed the greatest fall in temperature but all showed considerable fall. The rise in temperature was greatest in the normal, extract-treated, and DCA-treated adrenalectomized rats.

Normal rats, DCA-treated or extract-treated adrenalectomized rats that died usually reached a temperature of 40.5°C. or more. In a few cases a temperature

of 42°C. was recorded in a normal and the animal survived. Survival with a temperature of over 41°C. was not seen in the adrenalectomized animals.

The loss in body weight of all rats that survived was as follows: normal 7.8 per cent; untreated adrenalectomized, 5.2 to 6 per cent; 1 per cent NaCl-treated adrenalectomized, 8 to 8.8 per cent; DCA-treated adrenalectomized, 5.6 to 6.7 per cent; and extract-treated adrenalectomized, 5.9 per cent. It will be noted that normal and NaCl-treated adrenalectomized rats lost the most weight.

The water content of brain, blood, muscle and skin was determined in normal rats and adrenalectomized rats of the various groups with or without exposure to heat. Of the animals exposed to heat only those that survived were used. Since there was no significant difference between the three and four-day adrenalectomized animals, the two were combined. The results were based on eight to ten animals in each case, and are shown in table 3.

TABLE 2

Average temperatures of rats exposed to 38.7°C. for 4 hours

CONDITION	NUMBER OF ANIMALS	HOURS OF EXPOSURE					1 HOUR AFTER	RISE
		0	1	2	3	4		
Controls..	65	37.1°C.	38.8°C.	39.4°C.	39.9°C.	39.6°C.	36.6°C.	2.8°C.
Adrenalectomized								
No treatment. .	12	36.8°C.	38.9°C.	39.0°C.	39.0°C.	39.2°C.	36.3°C.	2.4°C.
1% NaCl-treated ...	29	36.8°C.	38.5°C.	38.8°C.	39.1°C.	39.3°C.	36.7°C.	2.5°C.
DCA-treated .	83	37.1°C.	38.7°C.	39.0°C.	39.5°C.	39.5°C.	36.1°C.	2.4°C.
Adrenal extract-treated..	26	36.9°C.	39.0°C.	39.2°C.	39.6°C.	39.5°C.		2.7°C.

There were no significant changes in water content of the brain in any of the animals. The untreated and sodium chloride-treated animals showed the greatest blood concentration. There was a loss of water from the skin of all of the animals, except those treated with DCA. The greatest loss occurred, however, in the normals. Water loss from muscle was either insignificant or small in all animals.

Discussion. The temperature to which the rats were exposed, 38.7± 0.1°, is only 1.6°C. above the average colonic temperature of the normal animals. A large proportion of the animals needed their adrenals to withstand this temperature for four hours. What is the function of the adrenals in this stress?

Let us review briefly the effects of exposure to heat and the differences in reaction between protected and unprotected animals. Salivation was much more copious in normal than in unprotected adrenalectomized rats. However, this could have had little effect on loss of heat in the humid atmosphere. The normal

or protected adrenalectomized rats were active and restless while the other rats became quiet and unresponsive. The colonic temperature of unprotected adrenalectomized rats did not rise as high as did that of the normal or the protected (by extract or DCA) adrenalectomized rats. This could be accounted for in part by the decreased activity. The muscular activity due to restlessness would facilitate the return of blood to the heart in the normal or protected adrenalectomized animals.

We made no blood pressure determinations but if rats react as Brenning (3) has shown for rabbits, the blood pressure of normal rats should eventually rise upon exposure to heat while that of adrenalectomized rats should not. At the beginning of the exposure unprotected adrenalectomized rats would have a sub-

TABLE 3

Percentage of water in tissue

TISSUE	CONTROLS	ADRENALECTOMIZED		
		No treatment	1% NaCl	DCA
Blood				
Before	81.62	81.42	81.66	81.33
After	80.78	77.73	77.58	80.31
% loss	1.03	4.53	4.99	1.25
Brain				
Before	78.79	78.69	78.71	78.79
After	78.30	78.96	78.73	78.52
Muscle				
Before	76.15	75.73	76.67	75.62
After	74.80	75.49	75.09	75.17
% loss	1.77		2.06	
Skin				
Before	63.69	62.06	64.93	62.77
After	59.15	60.65	62.69	62.89
% loss	7.12	2.27	3.45	None

normal blood pressure (4). This could be due to both cardiac (5) and vascular failure (1). In our experiments the unprotected adrenalectomized animals showed the greatest loss of water from the blood. This would indicate a reduction in blood volume and thus to that extent impair the circulation. The loss of water from the blood could be explained by a lessened ability to shift water from the tissues. Changes in the water content of the skin would indicate this, since the water loss there was much greater in normal rats than in the unprotected animals. In the DCA-treated adrenalectomized animals there was no loss of water from the blood and none from the skin.

The impaired circulation of the unprotected animal might fail to meet the increased demands produced by heat. The rapid shallow respiration might tend

to lower the oxygen tension in the alveolar air. Moreover, Banus (2) has shown that the oxygen capacity of the blood is reduced when the body temperature is maintáined at a high level for several hours. Under these conditions when increased circulation is needed heat causes the vessels to lose tonus and stasis may develop (7). In addition the high body temperature increases the oxygen requirement of the tissues, especially in the central nervous system. These changes occur in all of the rats, normals as well as adrenalectomized, but the normal and protected adrenalectomized animals are better prepared to compensate by circulatory adjustment.

Hartman, Lockwood and Lockie (6), several years ago, suggested that the increased suceptibility of adrenalectomized animals to heat was due to a failure of water mobilization. Their animals were exposed in a drying oven which would produce a very dry atmosphere and thus permit heat loss with the evaporating saliva from the coat of the rats. In the present study the high humidity of the atmosphere practically prevented such evaporation.

Adrenal extract and DCA protected adrenalectomized rats from heat equally well. The presence of DCA in the extract cannot account for this, since it occurs there only as a trace or not at all. Therefore, the substance which most nearly resembles it in physiological properties, viz., the sodium factor, may be responsible.

SUMMARY

1. Both normal and adrenalectomized rats, mostly male, weighing from 200 to 340 grams were exposed to a temperature of 38.7°C. for four hours in an atmosphere of high humidity.

2. Ninety-three per cent of the normal rats survived while only 14 to 17 per cent of the untreated adrenalectomized rats survived. Sodium chloride afforded some protection since 27 and 53 per cent of the adrenalectomized rats given 1 per cent sodium chloride to drink, survived. Treatment of adrenalectomized rats with desoxycorticosterone acetate was nearly adequate since 78 and 89 per cent survived. About the same survival was obtained with adrenal extract, namely, 89 per cent.

3. The normal or protected adrenalectomized rats were active and restless while the other rats became quiet and unresponsive.

4. The body temperature peak was reached during the third hour in the normal, DCA-treated adrenalectomized and extract-treated adrenalectomized animals and in the fourth hour in the others. The rise in temperature was greatest in the normal, DCA-treated adrenalectomized and extract-treated adrenalectomized rats.

5. Water content of brain, blood, muscle and skin was determined with and without exposure to heat. The untreated and sodium chloride-treated adrenalectomized rats showed the greatest blood concentration. There was a loss of water from the skin in all animals except the DCA-treated adrenalectomized rats, the greatest loss being in the normal animals. Salivation was more copious in the normal than in the unprotected adrenalectomized rats.

6. Since DCA protects as well as adrenal extract and acts most like sodium factor in its physiological properties it may be the sodium factor which is responsible for the protection afforded by adrenal extract.

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THE EFFECT OF ELECTRIC CURRENT ON GASTRIC SECRETION AND POTENTIAL[1]

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Studies have been undertaken in an attempt to answer the question as to whether electrical energy provides the energy for the production of osmotic work by living cells. The stomach was used as experimental material. Previously published results (3) demonstrated that there was no maintained depolarization of the potential in the resting stomach when the potential was shunted through a low external resistance. While the amount of electrical energy obtained by this method is not as large as that necessary for the production of the HCl of the gastric secretion, the fact that there is no maintained depolarization under these conditions indicates that the stomach is capable of producing greater amounts of electrical energy, perhaps a sufficient amount for the production of the HCl of gastric secretion. Other studies (4, 5) indicated that under certain experimental conditions there was a relationship (although a complex one) between the potential difference across the stomach wall and the rate of secretion of HCl. It was shown that a decrease in the potential difference across the secreting stomach following the application of relatively concentrated solutions of HCl was associated with a concomitant decrease in the secreting rate.

A further means of testing the above hypothesis and one that might be expected to yield information of a more crucial nature is a study of the effect of applied electric current on the production of HCl. A review of the literature reveals that several investigators have been interested in this problem. Their results, however, are conflicting and in the writer's opinion inconclusive because of inadequately controlled experimental conditions. In the most recent paper available to the writer Schawerin (7) concludes that the application of direct current has no effect on secretion. This paper should be consulted for a review of the earlier work. The primary purpose of the present work is a further investigation of the effect of applied electric current on gastric secretion. The potential difference across the stomach wall was also measured and certain correlations between the rate of secretion of HCl and the potential are presented.

METHODS. Dogs fasted for approximately 24 hours were anesthetized with pernoston[2] (80 mgm. per kgm., subcutaneously). A portion of the stomach along the greater curvature was placed in the chamber illustrated in figure 1. This chamber is a modification of the one described before (5). For a description of the technique for placing the stomach in the chamber this paper should be consulted.

[1] This investigation was aided by a grant from the Joseph E. Seagram and Sons Company.

[2] The Riedel-deHaen Company, New York, kindly supplied the pernoston used in these experiments.

The electrodes E_3 and E_4 were connected in series with lead storage batteries, a variable resistance, a milliammeter and the appropriate switches. The amount of current applied to the stomach was controlled by the variable resistance. Leads from electrodes E_1 and E_2 were connected to a potentiometer for measurement of the potential difference across the stomach. Electrode E_1 was connected with the fluid in the chamber (F) by a glass tube containing saturated KCl. Saturated KCl agar extended from electrode E_2 to the saturated KCl agar in the large lucite tube. Control experiments revealed that currents of the magnitudes used in the following experiments did not result in a significant change in the potential difference between electrodes E_1 and E_2 (change in potential difference less than one millivolt).

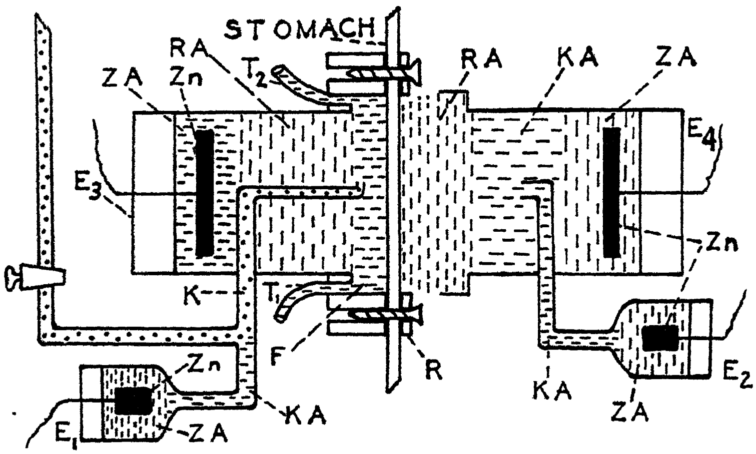

Fig. 1. Schematic drawing of a sagittal section of the apparatus with stomach in place. R refers to a lucite ring. This ring has an oblique cut in it so that the blood vessels (not shown) supplying the portion of the stomach in the chambers can be slipped inside it. RA refers to Ringer agar or saline agar; KA to saturated KCl agar; ZA to saturated zinc acetate agar; K to saturated KCl; F to the fluid in the chamber. Electrodes E_1 and E_2 are connected to a potentiometer for measurement of the potential difference across the stomach. Electrodes E_3 and E_4 were used for sending current through the stomach.

The secretory rate was measured in essentially the same manner as described before (5). Saline (0.9 per cent) was placed in the chamber and the chamber was drained and flushed out with more saline (enough to make a total sample of 100 ml.) every ten minutes (via tubes T_1 and T_2). Both the pH and the titratable acidity (using phenolphthalein as an indicator) were determined on each sample. The pH was usually measured within two minutes after a sample was obtained. In this way it was possible to determine when the rate of secretion of HCl had reached a relatively constant level. The milligrams of HCl secreted per ten minutes were calculated from both the pH and titration data. In experiments in which the glass electrode was calibrated before and after each pH measurement (with appropriate mixtures of 0.9 per cent NaCl and 0.159 N HCl) there was in the large majority of cases an excellent agreement between the

milligrams of HCl determined by titration and from the pH (see fig. 2). In some experiments in which this precaution was not taken there were greater discrepancies. It should be pointed out that in spite of these discrepancies calculations of the change in secretory rate from pH data and from titration data were in excellent agreement. It is obvious that in the absence of pH data an increase in the titratable acidity could not be considered proof of an increase in the secretion of HCl (the liberation of a buffered solution from the mucosa could result in an increase in the titratable acidity). The fact that changes in the secretory rate calculated from pH and titration data are in good agreement constitutes proof that in the following experiments the changes in the secretory rates are primarily the result of changes in the secretion of HCl.

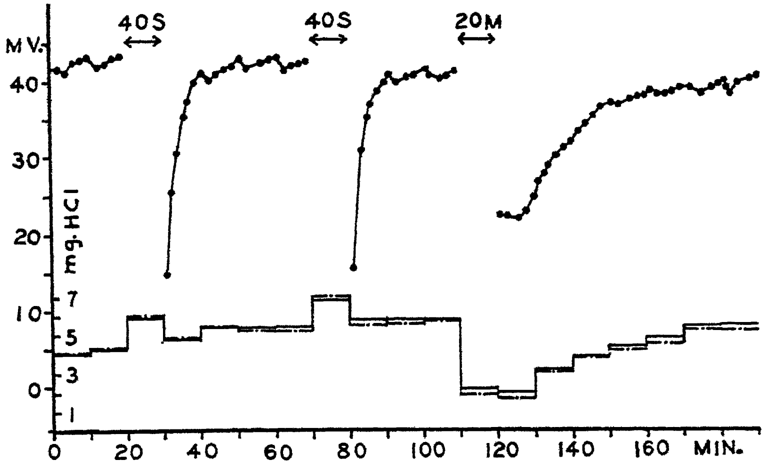

Fig. 2. Effect of applied electric current on potential and secretory rate. Solid dots represent potential in millivolts. Dash-dot lines represent milligrams of HCl secreted per ten minutes, as calculated from pH data; continuous lines represent milligrams of HCl as calculated from titration data. *40 S* represents ten minute periods during which 40 ma. were sent from serosa to mucosa; *20 M* represents a ten minute period during which 20 ma. were sent from mucosa to serosa.

Since, with the present technique, the fluid in the chamber is in contact with a relatively large surface of agar the following experiments were performed in an effort to determine how much of the HCl secreted by the stomach was neutralized or trapped by the agar. A cellophane membrane was used in place of the stomach and a known amount of HCl in 0.9 per cent saline (of slightly greater hydrogen ion concentration than that found in the chamber during secretion of HCl by the stomach) was introduced into the chamber and after a ten minute interval was removed in the same manner as that described for the obtaining of a sample. After another ten minutes the fluid was again similarly removed from the chamber. It was found that practically all of the HCl was recovered in the sample obtained after the first ten minute interval and only a small amount (less than $\frac{1}{4}$ mgm. of HCl) was present in the second sample. The effect of agar on the HCl in the chamber is, therefore, unimportant with respect to the results obtained in the following experiments.

The present technique has the distinct advantage over previous techniques (7) in that the current density is undoubtedly much more uniform. However, even with the present technique the current density is undoubtedly less at the periphery of that portion of the stomach in the chamber, since the serosal electrode did not make contact with this peripheral portion (approximately 2 mm.). A second chamber practically identical with the mucosal chamber (except that it contained an opening in the top for the blood vessels) was substituted for the serosal electrode in a number of experiments. With this two-chamber technique fluid was in contact with the total area of that portion of the stomach in the chamber and the current density would be expected to be more nearly uniform than with the above described technique. The results with the two-chamber technique were essentially the same as with the other technique and are, therefore, included in the following results without being separately designated.

In the following experiments the magnitude of the current represents the total amount of current applied to the stomach in the chamber. The area of the stomach in the chamber is 21 sq. cm. and the current density per square centimeter can be obtained by dividing by this figure.

RESULTS. *Effect of current applied to the secreting stomach on the secretion of HCl.* Histamine (usually 0.03 to 0.1 mgm. of the diphosphate per kgm.) was injected subcutaneously at ten minute intervals for the duration of an experiment. After the rate of secretion of HCl had reached a relatively constant level, electric current was applied. Experiments in which the duration of current flow was varied revealed that the passage of a current of appropriate magnitude for a ten minute period resulted in a readily detectable difference in the secretory rate. In all of the following experiments the current was applied for ten minute periods. The chamber was drained and flushed out with saline every ten minutes. The current was not turned on until the chamber had been drained and flushing with saline had been started (until the chamber had been refilled with saline) and was turned off immediately before the chamber was again drained for the next sample. The current did not, therefore, pass through the stomach for a full ten minute period, but for approximately twenty seconds less than ten minutes. In the following calculations on the effect of current no attempt was made to take into account the fact that the current was not turned on for a full ten minute period.

a. Effect of sending current from serosa to mucosa. A total of twenty-one experiments were performed in which current strengths of from 7 to 80 milliamperes were employed. Figure 2 represents the results of a typical experiment in which 40 ma. were sent for two periods from the serosa to the mucosa (the positive pole of the battery was connected to the serosal electrode E_4). It can be seen that there was a definite increase in the amount of HCl secreted during the period of current application. In figure 4A the increase in the secretion of HCl during the period of current application over that secreted during the preceding period is given for all of the experiments (solid dots). It can be seen that the increase in the secretion of HCl was roughly proportional to the magnitude of the current. For convenience of analysis (see below) the data were divided into two groups, the first group consisting of those experiments in

which 25 ma. or less were applied, and the second group including those experiments in which 40 ma. or more were applied. The average ΔS (increase in mgm. of HCl per ma.) for all of the experiments was 0.039 (S.E. ±0.0036). The average ΔS for the first and second groups was 0.046 (S.E. ±0.0098) and 0.037 (S.E. ±0.0031) respectively.

Inspection of the data revealed that, although current was not applied until a relatively constant secretory rate had been attained, there was an over-all tendency for the secretory rate to increase with time. The above values were, therefore, corrected for this tendency (the rate of change of secretory rate was taken as the difference in the secretory rates of the two periods preceding the application of current). After correcting for this factor the average ΔS for all the experiments was 0.030 (S.E. ±0.0063) and for the first and second groups 0.019 (S.E. ±0.022) and 0.036 (S.E. ±0.0043) respectively.

The above calculations take into consideration only the period of current flow and the preceding periods and, therefore, the conclusion would not be warranted on the basis of these data alone that the application of current results in an increase in the production of HCl. The increase in secretory rate during the period of current flow could conceivably result from the emptying of reservoirs in the mucosa containing already formed gastric juice. If this were the case then there should be a compensatory decrease in the secretion of HCl during the period following current flow. Inspection of the data revealed (in experiments in which currents of 40 ma. or more were used) that when the periods following current flow were also taken into account there was a total increase in the secretion of HCl. Inspection of the data also revealed that the secretory rate had reached a relatively constant level by the end of the first period following the application of current (the secretory rate of the second period following current flow was essentially the same as that of following periods). The total increase in the secretion of HCl was, therefore, calculated from the secretory rates of the period of current flow, the period following current flow and the two periods preceding current flow. The following formula was used.

$$\Delta S_T = S_C - (S_{1B} - S_{2B} + S_{1B}) + S_F - (2(S_{1B} - S_{2B}) + S_{1B})$$

where ΔS_T represents the total increase in HCl, S_C the secretory rate during the period of current flow, S_F the secretory rate during the period following current flow, S_{1B} and S_{2B} the secretory rates of the first and second periods respectively preceding the period of current flow (all of these values are in mgm. HCl). The average ΔS_T for all the experiments was 0.034 (S.E. ±0.024) and for the first and second groups 0.001 (S.E. ±0.075) and 0.052 (S.E. ±0.009) respectively.

It can be seen from these figures that the application of currents of 40 ma. or more results in not only an increase in the secretory rate during the period of current flow, but also in a total increase in the secretion of HCl as defined above. On the other hand the results of the application of 25 ma. or less are not conclusive. It should be pointed out that most of these latter experiments were performed during periods (early in a series of experiments on a given dog) in

which there was a relatively greater tendency for the secretory rate to change with time. The large standard errors in these experiments can be attributed to the fact that these changes in secretory rate were large compared to the changes induced by the current.

b. Effect of sending current from mucosa to serosa. Figure 2 represents the result of a typical experiment in which 20 ma. were sent for one period from the mucosa to the serosa (the positive pole of the battery was connected to the mucosa electrode E_3). It can be seen that the application of current resulted in a decrease in the secretion of HCl. The results of all of the experiments in which current was applied from the mucosa to the serosa to an actively secreting stomach are shown in figure 4A. The open circles represent the difference in the amount of HCl secreted between the period of current flow and the preceding period.

In figure 2 it can be seen that the secretory rate was depressed after the current was turned off, and that it gradually recovered (recovery had started by the second period following application of current) over a period of about one hour to approximately its former magnitude. This was found to be essentially true in those experiments in which 25 ma. or less were applied to the stomach. Application of 40 milliamperes or more from mucosa to serosa usually resulted in a much more prolonged and marked depression of the secretory rate (usually for the duration of the experiment, 40 to 80 min.). This is illustrated in figure 3A. In this experiment there was a relatively marked depression of the secretory rate with little tendency toward recovery for the following 40 minutes. In the light of the above, the total decrease in the output of HCl when current was sent from the mucosa to the serosa was much greater than that represented in figure 4A, since the values given in this figure are for the difference in secretory rate during just two periods.

It is obvious from the above that applied current produces a much greater total decrease in the secretory rate as a result of sending current from mucosa to serosa than increase in the secretory rate from sending the current in the opposite direction.

Effect of current applied to the non-secreting stomach on the secretion of HCl. Experiments similar to the ones described above were performed on the non-secreting stomach. Sixteen experiments were performed in which current was sent from serosa to mucosa. The range of current strength in these experiments was from 7 to 150 milliamperes. Eight experiments were performed in which current was sent from mucosa to serosa and the current range was from 10 to 125 milliamperes. In none of these experiments was there any evidence that application of current resulted in a secretion of HCl as determined by changes in the pH of the samples. Sometimes, however, particularly with the higher currents, a small increase in the titratable acidity resulted either during the period of current application, as seen in figure 3B, or following the period of current application, as seen in figure 4B. Since there was no appreciable decrease in the pH, this small increase in the titratable acidity is probably due to liberation from the mucosa of substances other than HCl.

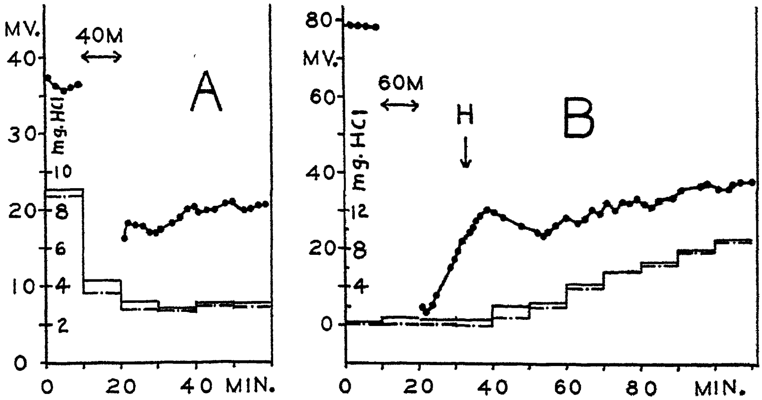

Fig. 3A. Effect of 40 ma. sent from mucosa to serosa on secretory rate and potential.

Fig. 3B. Effect of 60 ma. sent from mucosa to serosa on secretory rate and potential of the non-secreting stomach. At time indicated by arrow marked *H*, histamine diphosphate was given subcutaneously and, as in all of the experiments, it was repeated at ten minute intervals for the duration of the experiment.

Conventions same as in figure 2.

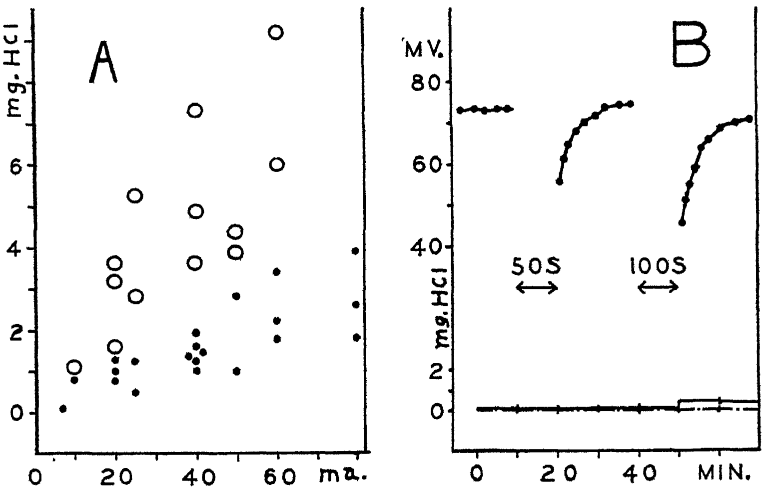

Fig. 4A. Relationship between change in secretory rate in milligrams of HCl and current strength in milliamperes. Solid dots represent increase in secretory rate as a result of sending current from serosa to mucosa. Open circles represent decrease in secretory rate as a result of sending current from mucosa to serosa (see text).

Fig. 4B. Effect of current sent from serosa to mucosa on the non-secreting stomach. Same conventions as in figure 2; *50 S* and *100 S* respectively, represent periods during which 50 ma. and 100 ma. were sent from serosa to mucosa.

Correlation between potential and secretory rate following application of current. In the present experiments no attempt was made to measure the potential difference across the stomach during the application of current or during the first minute after the current was turned off.

It can be seen from figures 2, 3 and 4 that one minute after the current was turned off the magnitude of the potential was less than before the application of current in both the secreting and non-secreting stomach, irrespective of the direction of the applied current. This was true for all experiments in which the current was sent from serosa to mucosa in both secreting and non-secreting stomachs. It was also true for all the experiments in which more than 25 milliamperes was sent from mucosa to serosa in both secreting and non-secreting stomachs. However, when currents of 25 ma. or less were sent from mucosa to serosa there was in some experiments an increase in the potential one minute after the current was turned off.

In the experiments in which current was sent from serosa to mucosa there was a comparatively rapid return of the potential to its original level, as can be seen from figures 2 and 4B. On the other hand, following the sending of current from the mucosa to the serosa there was a more prolonged depression of the potential, as can be seen in figures 2 and 3.

The recovery of the potential, following the depression resulting from sending current from the mucosa to the serosa, closely parallels the recovery of the secretory rate, as can be seen in figure 2. A maintained depression of the potential (fig. 3A) is always associated with a maintained depression of the secretory rate. In none of the experiments was there ever recovery of the secretory rate without a concomitant recovery of the potential. On the other hand, in two experiments there was partial recovery of the potential without recovery of the secretory rate.

Figure 3B shows a typical experiment in which the effect of histamine on the potential and secretion is shown after the potential had been previously depressed by applying current from the mucosa to the serosa. It can be seen that with the onset of secretion the gradual increase in potential was reversed, and that as the secretory rate increased the potential again increased and approached the magnitude of potential found in secreting stomachs not initially depressed by applied current.

In the present series of experiments data were incidentally obtained on the effect of histamine on the resting potential of eighteen dogs. The results in sixteen of these dogs were quite similar (resting potential above 70 mv.) to those reported previously (5). However, in two dogs the resting potential did not rise above 50 and 54 mv. No evidence of a resting secretion of HCl was obtained. After histamine stimulation the potentials dropped to the neighborhood of 40 mv. in both dogs and the amount of secretion was within normal limits.

Discussion. The findings of the present work offer additional support to the hypothesis that electrical energy furnishes the energy for the production of HCl. This is the first time as far as the writer is aware that it has been demonstrated that a secretory process could be augmented or decreased by application in the appropriate direction of a direct current. It is obvious that much more work has to be done before anything approaching a final interpretation can be placed on these findings. However, the present experiments serve as relatively good controls for each other. For example, the increase in the secretion of HCl

resulting from the passage of current from the serosa to the muccosa is probably not due to stimulation of secretory nerves since application of currents to the resting stomach did not produce secretion. The finding that application of current to the resting stomach did not result in secretion can be interpreted (in light of the electrical energy hypothesis) as indicating that the characteristics of the stomach must be modified by histamine (or some other gastric stimulant) before electrical energy is effective in producing HCl. It is possible that the electrical resistance of certain portions of the mucosa may be quite different in the resting and secreting stomach, and that the amount of current passing through these regions may for a given total current be much less in the resting than in the secreting stomach.

Further evidence has been presented that a correlation exists between the magnitude of the potential and the secretory rate. Depression of the potential of the secreting stomach following application of current from mucosa to serosa was always associated with a depression in the secretory rate. These experiments are strikingly similar to those (5) in which the potential and secretory rate were depressed following the application of HCl to the mucosa of the secreting stomach.

The minimum amount of free energy necessary for the production of the inorganic ions of the gastric juice can be calculated, and it would therefore be pertinent to determine the efficiency of the increase in the production of HCl as a result of applying current from the serosa to the mucosa. Bull (see Gray (2)) and Davenport and Fisher (1) have published such calculations; the former reported 1,531 calories, and the latter 820 calories per liter of gastric juice. The writer's calculations (6) are in essential agreement with those of Bull and, therefore, 1,531 calories will be used in the following. Bull made his calculations on the assumption that one liter of gastric juice contained 0.159 M of HCl. On the basis of these calculations the minimum amount of free energy needed for the production of 1 mgm. of HCl is 0.26 calorie or 1.1 joules. The electrical energy absorbed by the stomach when current was sent from serosa to mucosa would be equal to $RI^2t + EIt$, where R is the resistance of the stomach in the chamber in ohms, I the magnitude of the applied current in amperes, E the potential difference across the stomach in volts, and t the time in seconds. An average value for R was taken from previous data on the resting stomach (3). It was assumed that the total resistance across the stomach wall is not markedly different in the secreting and non-secreting stomach (6). Calculations with the above units give the energy in joules. It was found that the total electrical energy delivered to the stomach was greater (markedly so for currents of 40 ma. or more) than the amount of energy needed for the formation of HCl. However, the RI^2t energy is converted into heat and only a negligibly small fraction of this heat could be theoretically converted into useful work (assuming reasonable values for temperature gradients in the stomach). On the other hand, the EIt energy could be theoretically used for useful work. This energy was calculated assuming E = 0.05 volt (see previous paper (5) in which evidence is presented indicating that, after elimination of the diffusion potential of the gastric juice into the saline of

the chamber, the gastric potential lies somewhere between 40 and 60 mv.). This energy was found to be (EIt = 0.05 volt × 0.001 ampere × 600 sec.) 0.030 joule per ten minutes per ma. Unpublished evidence (6) warrants the assumption that the potential is not decreased during the flow of current since the potential immediately after the current is turned off is not markedly different from its value before application of the current (the potential then drops rapidly during the first minute). The average total increase in the secretion of HCl was taken as 0.052 mgm. HCl per ma.

On the basis of the above assumption 0.03 joule of electrical energy can produce 0.052 mgm. HCl. Assuming that the concentration of HCl in gastric juice secreted under the conditions of these experiments is 0.159 N, it was found that approximately 800 calories would be required for the production of one liter of 0.159 N HCl. Since energy is never converted from one form to another with an efficiency of 100 per cent, it would actually require more than 1,531 calories for the production of one liter of gastric juice. The finding that one liter of 0.159 N HCl would be produced by 800 calories would indicate that either the curent acts, at least in part, as a trigger mechanism or that some other process furnishes part of the energy necessary for the production of HCl. For example, if some other process lowers the pH in some region of the gastric mucosa (see Davenport's theory (1)), the amount of free energy necessary for the production of the HCl of gastric juice from this fluid of lowered pH would be less than 1,531 calories. If the pH of this fluid was 4.4 then the minimum free energy needed to form one liter of 0.159 N HCl would be approximately 800 calories. However, since practically nothing is known about the internal circuits of the stomach, the amount of the EIt energy absorbed at any particular locus might be greater or smaller than that calculated above. It is possible that the value of E may be greater than the value assumed above (0.05 volt) during the actual flow of current. Also it should be pointed out that, on the basis of other correlations between the secretory rate and potential, the actual production of HCl during a portion of the period following the application of current would be expected to be depressed since the potential is depressed for part of this period. The secretory rate, however, during this period is greater in the experiments of 40 ma. or more than that expected if no current had been applied. Some of the HCl produced during the period of current application is probably not secreted until the following period, and it would be possible for the secretory rate during this period following current flow to be greater than that expected (if no current had been applied) even though there was a decrease in the production of HCl during this period. Therefore, the increased amount of HCl produced during the period of current flow might be greater than that calculated, i.e., 0.052 mgm. per ma.

In the light of the above discussion it is not possible at present to come to a definite conclusion on the quantitative relationship existing between the amount of electrical energy absorbed at particular loci inside the stomach capable of doing useful work and the increase in the amount of HCl produced. However, the above analysis indicates that the electrical energy absorbed by the stomach capable of doing useful work is probably of the same order of magnitude as the amount needed for the production of the HCl of gastric juice.

There are many implications of the results presented above that cannot be gone into here. Obviously, much more work needs to be done on the electrophysiology of the stomach before the hypothesis presented in the introduction can be established or disproved. So far the data presented in this paper and preceding ones offer evidence in support of this hypothesis.

SUMMARY

1. Electric current applied from the serosa to the mucosa of the secreting stomach results in an increase in the secretion of HCl. The minimum free energy necessary for the production of this increase in the amount of HCl is of approximately the same order of magnitude as the electrical energy delivered to the stomach that is capable of doing useful work (see Discussion).

2. Electric current applied from the mucosa to the serosa of the secreting stomach results in a decrease in the secretion of HCl. The total decrease in the production of HCl resulting from application of current from mucosa to serosa is much greater than the increase in the secretion of HCl resulting from the application of current from serosa to mucosa.

3. Electric current applied from the serosa to the mucosa or in the opposite direction to the non-secreting stomach does not result in the secretion of HCl.

4. Depression of the potential resulting from the passage of current from mucosa to serosa was associated with a depression of the rate of secretion of HCl. Recovery of the secretory rate was always associated with a concomitant recovery of the potential.

5. Histamine stimulation, following the depression of the potential resulting from the application of current from mucosa to serosa in the non-secreting stomach, resulted in secretion and was associated with a relatively small change in the potential. In these experiments the level of the potential after secretion was well established was essentially the same as that found in secreting stomachs not initially depressed by applied current.

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ANTICHOLINESTERASE ACTIVITY OF ACID AS A BIOLOGICAL INSTRUMENT OF NERVOUS INTEGRATION[1]

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In our studies on humoral mediation in the central nervous system we have found that an intravenous injection of eserine produces a prolonged increased activity of the respiratory center. This is attributable to a potentiation of normal respiratory reflexes by the anticholinesterase activity of eserine (Gesell and Hansen, 1943). This observation, in conjunction with the facts that acids (carbonic and lactic) are formed in great and varying abundance as the end products of aerobic and anaerobic metabolism and that the anticholinesterase activity of acid increases with cH (Glick, 1937), constitutes the basis for our belief that physiological fluctuations of cH function as an instrument of nervous integration (Gesell, Brassfield and Hamilton, 1942). The anticholinesterase activity of acid serving as a complementary mechanism to the physiological fluctuations of synaptic bombardment provides a dual mechanism for determining the intensity of nervous activity. As Sherrington has shown, an increase of strength or duration of sensory stimulation intensifies spinal reflexes and prolongs the duration of their after-discharges. These results harmonize with cholinergic motivation of nerve cells, since the amount of endogenously liberated acetylcholine surviving destruction should increase, within limitations set by the mechanism, with an increasing sum total of bombardment (see Cannon and Rosenblueth and their associates, and others). Yet contrary to the firmly established findings of Sherrington on spinal reflexes, the after-discharge of respiratory reflexes (after-hyperpnea) to faradic stimulation of Hering's nerve (sinus nerve) is shortened rather than lengthened by a prolongation of sensory stimulation.[2] This disagreement of fact and theory, however, is more apparent than real, if it be assumed that the excessive ventilation which alkalinizes the body increases the cholinesterase activity and thereby augments the destruction of acetylcholine. The speedier lowering of the acetylcholine pools occurring in the respiratory center thus hastens the subsidence of breathing and reverses the classical observations of Sherrington (1906).

This view is now supported by readily demonstrable potentiating effects of acid on the response of several cholinergic systems to extrinsic acetylcholine (the heart of the turtle, the respiratory muscles of the frog, turtle and alligator, the submaxillary gland of the dog, and the gut of the rabbit; Brassfield and Gesell, 1942; Gesell, Mason and Brassfield, 1944a, b). It is therefore postulated that

[1] Preliminary reports—Proceedings 1942.

[2] It should be remembered that Hering's nerve contains inhibitory afferents from the sinus and excitatory afferents from the chemoceptors. Excitatory effects dominate with the strength of stimulation employed in our experiments.

the main effect of acid, similar to that of eserine on the nervous integration of the respiratory act, lies in its anticholinesterase activity. Though non-stimulating in itself the end effect of acid is also an increased motivation of nerve cells. Just as the amount of power accumulated by a hydraulic dam is determined by the volume flow of the converging streams and the height of the dam conserving the water, so is the amount of nervous power available for the motivation of the brain determined by the height of the dam (i.e., the intensity of the anticholinesterase activity) and the volume of the converging streams of impulses. Working hand in hand with the physiological fluctuations of synaptic bombardment, physiological fluctuations in cH are thought to control the level of the pools of nervous force which propel the respiratory center. Our study inquires into the probability of these conjectures. Though our present experiments are limited to observations on the respiratory act the question of a broader application to neurophysiology in general is raised.

METHODS. The method of study consists in recording the effects of sensory nerve stimulation on breathing under varying conditions of acid-base equilibrium. Two types of nerves were found particularly useful: Hering's nerve, and cutaneous sensory nerves which increase the respiratory minute volume, and the cervical vagus and superior laryngeal which diminish pulmonary ventilation and hold respiration in the expiratory phase. The cH of the tissues was controlled by administration of carbon dioxide in oxygen-containing mixtures, by adjustment of the volume of artificial ventilation, and by stimulation of sensory nerves selected either to increase or decrease the respiratory volume. Breathing was recorded with a Hutchinson spirometer connected with rebreathing tanks. During artificially produced pneumothorax the intensity of respiratory movements was registered with the aid of recording bands encircling the torso. In some instances the degree of change of arterial cH was continuously recorded with the glass electrode. We are indebted to Doctor Brassfield for these recordings. All observations were made on dogs, most of which were anesthetized with morphine and urethane.

RESULTS. *A comparison of the effects of increased cH on the activity of the respiratory center with the response of the rectus abdominis muscle to acetylcholine.* When an anesthetized dog is caused to rebreathe a gaseous mixture of carbon dioxide, oxygen and nitrogen, the volume of breathing increases progressively as the tissues turn more acid (see fig. 1C). The rectus abdominis muscle of the frog, when placed in a weak solution of acetylcholine, records a slowly rising curve of contraction bearing a striking resemblance to that of increasing hyperpnea during progressive hypercapnia (see fig. 1D). Acidification of the acetylcholine solution with carbon dioxide accentuates the gradient and increases the final height of contraction (see fig. 1E). This comparison of breathing and muscular contraction is of interest in that the respiratory center of the dog and the rectus abdominis muscle of the frog are cholinergic systems of widely differing structure and organization. The respiratory center is a relatively complex unit consisting of a large variety of neurons structurally and functionally connected with one another. The isolated rectus abdominis muscle is, on the other hand,

an exceedingly simple structure composed of units of muscle fibers and corresponding motor end plates, structurally isolated and functionally insulated from one another.

The similarity of the response of the respiratory center to carbon dioxide, of the rectus abdominis to acetylcholine and to acetylcholine plus carbon dioxide

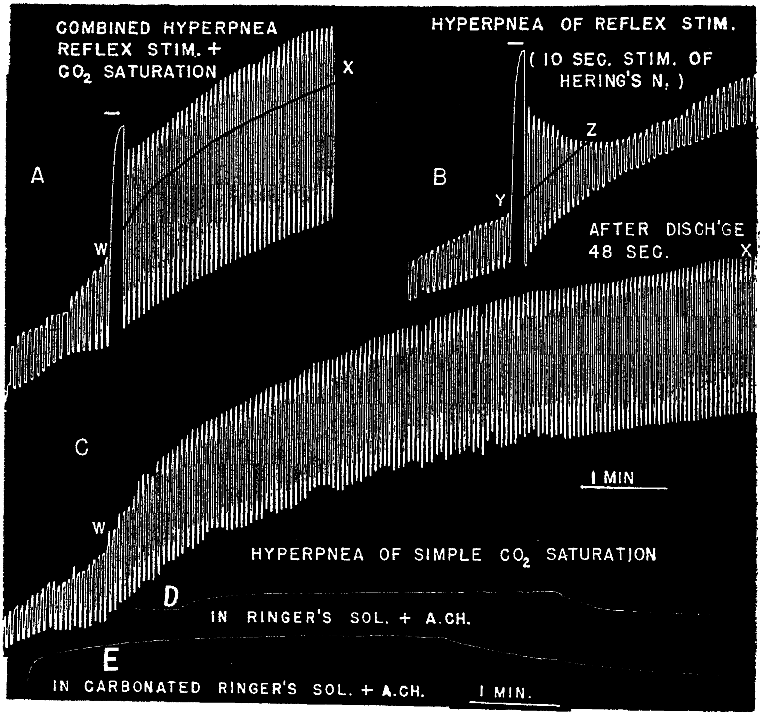


Fig. 1. The potentiation by carbon dioxide of the stimulating action of endogenous and exogenous acetylcholine. C shows increasing hyperpnea produced by the administration of carbon dioxide (potentiation of the stimulating action of endogenous acetylcholine liberated by the basal bombardment of the respiratory center). This is to be compared with D and E which show a progressively increasing tetanic contraction of the rectus abdominis muscle placed in a solution of exogenous acetylcholine. D, non-acidified and E, acidified solutions. A and B show the after-discharge produced by endogenous acetylcholine liberated by artificial stimulation of Hering's nerve during hypercapnia and during breathing or room air. The after-discharges are represented by the record lying above line WX and YZ respectively. The slow relaxation of tetanic contraction after the termination of immersion represents after-discharge of muscle to lingering exogenous acetylcholine. Note that the after-discharge is larger in E than in D.

illustrated in curves 1C, 1D and 1E suggest that similar forces are at work in these two systems. Electrical records show that when a rectus abdominis muscle is submerged in an acetylcholine-containing solution its fibers contract rhythmically at varying individual frequencies (Finerty and Gesell, unpublished data). The curve of contraction which the muscle records in figure 1D is therefore a resultant of summed contractions of a large number of fibers twitching

independently of each other. The slowly increasing strength of contraction is explained by the progressive penetration of acetylcholine into the muscle and a consequent addition of more and more muscle fibers to the contraction. When carbon dioxide enters the muscle along with acetylcholine as in figure 1E the muscle is acidified and the accompanying acetylcholine is better protected against hydrolysis. Acetylcholine consequently penetrates more rapidly and reaches a greater number of motor end plates in equivalent time, augmenting the gradient and intensity of contraction.

If it be granted that the act of breathing is fundamentally a reflex phenomenon dependent upon synaptic bombardment the explanation of the gradual development of hypercapnic hyperpnea becomes analogous to that of the progressively increasing intensity of contraction of the rectus abdominis muscle subjected to exogenous acetylcholine and to acetylcholine plus carbon dioxide. While the animal is at rest under anesthesia it may be assumed that the synaptic bombardment of the respiratory center, the associated liberation of free acetylcholine and the hydrolysis of free acetylcholine all remain uniform. Consequently the amount of free acetylcholine driving the respiratory center remains constant and breathing goes on at a steady pace. On breathing carbon dioxide, however, this balance of liberation and destruction of acetylcholine is disturbed. As the center grows more acid, less of the liberated acetylcholine is hydrolyzed, therefore more accumulates, neurons are activated in greater numbers and many are driven harder.

Heymans (1939) observed that the respiratory center itself is highly sensitive to carbon dioxide and Gesell and Moyer (1937) have shown that the chemoceptively-denervated dog responds to hypercapnia in much the same manner as does an intact animal. Gesell, Lapides and Levin (1940) have demonstrated that a sudden removal of the chemoceptive support of the carotid bodies attained by bilateral blocking of Hering's nerve (after previous elimination of the chemoceptive support of the aortic bodies by double vagotomy) produces little or no reduction of hyperpnea during rebreathing of carbon dioxide mixtures of 6 or more per cent. Since the vagus nerve and Hering's nerve were cut on both sides in figure 1C the hyperpnea produced by rebreathing carbon dioxide may be assumed to originate in the central action of carbon dioxide. This action, if the acid-humoral concept is sound, is not one of direct stimulation of the center but of a conditioning of the center allowing better conservation and employment of physiologically-liberated acetylcholine.

The correctness of this interpretation may be tested by noting the effects of hypercapnia on the response of the respiratory center to artificially-created bombardment added to the prevailing basal bombardment. More specifically, will artificial superbasal bombardment be potentiated by the action of carbon dioxide as physiological basal bombardment seems to be? The answer to this question is indicated in a comparison of figures 1B and 1A in which Hering's nerve is stimulated for periods of 10 seconds; figure 1A shows the response to stimulation during the administration of a carbon dioxide mixture while the nerve cells are turning more acid and more capable of preserving their endogenous supplies

of acetylcholine. Breathing increases tremendously on stimulation and remains at a high volume for an indefinite period after stimulation has ended. Line *WX* obtained by superimposing figure 1C upon figure 1A shows the increase in hyperpnea which was to have been expected during simple saturation of the dog with carbon dioxide in the absence of artificial superbasal bombardment produced by electrical stimulation of Hering's nerve. The area of the respiratory record above line *WX* therefore approximates the magnitude of after-discharge to this momentary bombardment. The persistence of the hyperpnea in figure 1A stands in marked contrast to the brief and rapidly diminishing after-discharge to a similar stimulation during normal conditions of eupnea represented in the area above line *YZ* in figure 1B.

It should, however, be realized that a measurement of the after-discharge to the acetylcholine laid down by the artificial bombardment during a progressive saturation of the animal with carbon dioxide can only be approximate, as will be more apparent in the following section. In the first place, the brain is turning more acid and therefore potentiation of the basal bombardment must increase. Secondly, the bombardment of the respiratory center is actually increasing due to the growing volume of proprioceptive bombardment originating in the augmenting respiratory movements. On first sight figure 1A suggests that the effects of the artificial bombardment are perpetually sustained so long as hypercapnia continues. This, however, implies that hydrolysis of acetylcholine has been completely stopped. If that were true, all of the acetylcholine liberated by physiological bombardment must accumulate and soon lead to paralysis of the centers from over-concentration of the neurohumor.

Comment on the nature of after-discharge. It has been suggested in an earlier publication that the repetitive twitching of muscle fibers subjected to exogenous acetylcholine is a rhythmic response to an electrotonic current set up at the motor end plate (see fig. 11 and also Gesell, Mason and Brassfield, 1944). As is illustrated in figure 1C the summed contraction of these twitches continues for a minute or more after the acetylcholine solution surrounding the muscle has been replaced by Ringer's solution. Contraction finally ceases when the acetylcholine concentration at the motor end plates has fallen below threshold values. This represents a very simple example of after-discharge. The after-discharge is markedly prolonged in figure 1E where hydrolysis of acetylcholine is retarded by the action of carbon dioxide. The comparison of after-discharge in figures 1D and 1E gives the clue to a simple interpretation of the duration of after-discharge of the respiratory center to stimulation of Hering's nerve during eupnea and hypercapnia in figures 1B and 1A.

According to the electrotonic theory of nerve cell motivation as originally outlined by Gesell (1939, 1940a), the after-discharge produced by sensory stimulation cannot be explained by circulation of nerve impulses in a circuitous chain of neurons as proposed by Lorente de No, Ranson, and others. The electrotonic theory demands another explanation, for it holds to the view that the neuron functions in three distinct capacities: 1, as a generator of nerve impulses; 2, as a conductor of these impulses, and 3, as a motivator; the dendrites and cell body

act as *generators*, the neuraxons as *transmitters*, and the end plates as motivators of other nerve cells or of end organs such as muscle fibers (see figs. 10 and 12). Acetylcholine liberated at each activated synapse is assumed to produce local negativities which adding to one another establish a common stationary electrotonic current (internal circuit—dendrites to cell body to axon hillock; external circuit—axon hillock back to dendrites. See fig. 9). The steady action of this dendrite-cell body current, concentrating and emerging at the axon hillock detonates the hillock and sets up a rhythmical series of *new* impulses which are conducted from the hillock to but not beyond the terminal synapses of its respective neuraxon. This topographical specialization of function is regarded as a basic key to nervous integration for it implies that nerve impulses do not travel through a chain of neurons. Transmission being limited to the neuraxon proper, nerve impulses cannot circulate within the central nervous system.

Nor is the circus theory essential for the explanation of the after-discharge of centers since simple preparations devoid of circuitous nervous paths are capable of striking peripheral after-discharge (twitching of striated muscle: Brown, Dale and Feldberg, 1936; Brown and Harvey, 1938; Gesell, Mason and Brassfield, 1944; after-inhibition of the isolated heart, and after-discharge of sympathetic ganglia: Bronk and Larrabee, 1937). With such positive evidence at hand, a continuing activity of a reflex arc after sensory stimulation has ceased is readily accounted for by combined and simultaneous electrotonic after-activities of the individual nerve cells comprising the arc, in which the neurons pass their tonic after-activity from one nerve station to another. Gesell, Magee and Bricker (1940), for example, have recorded the characteristic slowly augmenting inspiratory pattern at many points along the inspiratory arc (diaphragm and other respiratory muscles, phrenic nerve, ventral horn cell, reticulo-spinal tracts, medial reticular formation, lateral reticular nucleus, internal arcuate fibers, nucleus cuneatus and gracilus, parafasicular gray, and vagus nerve). By means of relay "nervous pressure" rather than nerve impulses is thought to be passed along the arc.

Effects of hypocapnia on the respiratory center. If hypercapnia promotes an increased volume of breathing by virtue of an increased anticholinesterase activity at the respiratory center, hypocapnia should produce a diminished volume of breathing by virtue of a decreased anticholinesterase activity. The effects of hypocapnia are conveniently studied by administering suitably graded pulmonary ventilation during double pneumothorax and recording the respiratory movements with the aid of torsal bands made of paper which encircle the chest and abdomen and record the changes in circumference of the torso. In figure 2 a highly excessive ventilation beginning at *B* is attended by a rapid alkalinization of the body and a correspondingly rapid disappearance of the respiratory movements. Apnea occurred within 35 seconds after hyperventilation was started. However, when artificial ventilation is adjusted to a volume only slightly greater than that demanded by the basal needs of the animal, the respiratory movements subside more slowly, presumably because such ventilation washes out the carbon dioxide more slowly (see fig. 4C) a presumption con-

firmed with the glass electrode. When apnea is reached, whether by rapid or slow alkalinization, the endogenous acetylcholine liberated by physiological bombardment can no longer accumulate in sufficient quantity to stimulate the respiratory neurons, i.e., the acetylcholine pools are believed to have receded from their preceding eupneic levels and dropped to sub-threshold or apneic levels.

Theoretically the effects of superbasal bombardment of the respiratory center should be reduced by a lowered cH just as are the effects of basal bombardment. Figure 2 illustrates a reflex response of the center to a short period of faradic stimulation of Hering's nerve delivered at point C during hypocapnia while the neurons are losing their power of conserving their endogenous supplies of acetylcholine. Excessive artificial ventilation begins at *B*. Shortly after apnea is established Hering's nerve is stimulated at *C*. Though the center was capable of reacting, the response was extremely short, barely longer than the period of stimulation. Only one deep breath occurred and that breath occupied the full

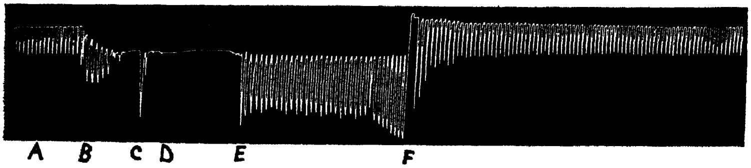


Fig. 2. A comparison of the effects of hypo and hypercapnia on the response to stimulation of Hering's nerve. Breathing is recorded with the aid of a costal band. A. Normal breathing with the chest intact. B. Double pneumothorax plus excessive ventilation leading to a rapidly developing hypocapnic apnea. C. Response to 4 seconds' stimulation of Hering's nerve (virtually no after-discharge). D. Resaturation of animal with 10 per cent CO_2 mixture. Before apnea had terminated Hering's nerve was stimulated again at E. The response is more vigorous and the after-discharge is prolonged. F. Pneumothorax terminated after which animal breathes room air.

period of stimulation. The single small breath which follows, therefore, represents the entire after-discharge, a result differing strikingly not only from that of figure 1A during hypercapnia but from that of figure 1B during eupnea, when the tissues of the animal are at their normal cH. In order to turn the animal more acid again carbon dioxide was administered at *D* in figure 2 shortly after stimulation of Hering's nerve. Just before apnea was about to terminate Hering's nerve was stimulated again at *E*. Breathing began immediately and with a slightly stronger contraction than that of eupnea. The excursions also remained higher than at *A*, indicating that an after-discharge had been set up as a result of the re-acidification of the center.

As judged by the reflex responses of the respiratory center to artificial sensory stimulation during hyper and hypocapnia we may conclude in agreement with our introductory statements that the magnitude of breathing is importantly related to two factors: A, the volume of synaptic bombardment or the rate at which endogenous acetylcholine is liberated at the respiratory center, and B, the rate of destruction of this acetylcholine as determined by the relative in-

tensity of cholinesterase and anticholinesterase activity. During eupnea both factors tend to remain uniform while during progressive hyper or hypocapnia both factors tend to vary. Hypercapnia increases anticholinesterase activity and the volume of synaptic bombardment. Extra or superbasal bombardment of the respiratory neurons originates in at least three sources: 1, an increasing chemical stimulation of the chemoceptors; 2, an increasing inflow of muscular proprioceptive impulses arising from intensified contractions and extensions of the respiratory muscles, and 3, a greater bombardment by the pulmonary proprioceptive impulses originating in augmented inflation and deflation of the lungs, since the vagus nerves have a dual excitatory influence, driving both the inspiratory and the expiratory centers (see Gesell, 1940a for heterogeneous bombardment of the respiratory center, and figs. 9 and 13).

In contrast to hypercapnia, hypocapnia tends to diminish the intensity of anticholinesterase activity and the volume of bombardment of the respiratory center in proportion to the lowering of cH and the withdrawal of chemoceptor, muscle proprioceptive and pulmonary proprioceptive support.

It is pointed out that a blowing off of carbon dioxide or an administration of cholinesterase should produce comparable effects on naturally prevailing reflexes. The experiments of Mendel and Hawkins (1943) showing a weakening of ocular reflexes after intravenous injection of cholinesterase give support to this general line of reasoning.

A comparison of respiratory and spinal reflexes. Respiratory and non-respiratory motor reflexes have the purpose of adjusting the intensity of muscular contractions. This similarity of purpose implies a similarity of mechanisms for attaining the common end effects. On the other hand, spinal and respiratory reflexes differ widely in the functions they are designed to control, which in turn implies a possible difference of integration in which the factor of cH might play no rôle in the non-respiratory reflexes. So with the discovery that the duration of respiratory reflex after-discharge is shortened rather than lengthened by a prolongation of sensory stimulation, as described by Sherrington for spinal reflexes, a more rigid analysis becomes mandatory.

If it is true that the shortening of after-hyperpnea produced by a prolongation of stimulation of Hering's nerve is due to a washing out of carbon dioxide from the tissues (Gesell, Brassfield and Hamilton, 1942) the effects of a prolongation of stimulation should be reversed if alkalinisation is prevented and thus conform with the findings of Sherrington. This proves to be true in figure 3 where Hering's nerve is stimulated 1, 5, 10, 20 and 40 seconds respectively. The chest is intact which allows reflex hyperventilation of the lungs. In figures 3A, B and C where the periods of stimulation were relatively short—1, 5 and 10 seconds respectively—the magnitude and the duration of the after-hyperpnea increased progressively. But comparing figures 3C, D and E where the periods of stimulation were 10, 20 and 40 seconds respectively the magnitude and duration of the after-hyperpnea are found to diminish. This extraordinary shift in results from a lengthening to a shortening of after-discharge with increasing duration of stimulation is attributed to the progressively increasing effects of

hyperventilation. Where only one deep breath occurs during the period of stimulation as in figures 3A and B obviously no alkalinization of the respiratory center could possibly occur during the course of stimulation and extremely little, if any, after the cessation of stimulation. In figures 3D and E, however, where a marked hyperpnea continues for 20 and 40 seconds respectively, alkalinization of the respiratory center undoubtedly does occur as soon as the hypocapnic blood produced by overventilation of the lungs reaches the brain. The turning point of the effects of prolongation of stimulation presumably comes at the moment when the strengthening effects of an increasing accumulation of acetylcholine

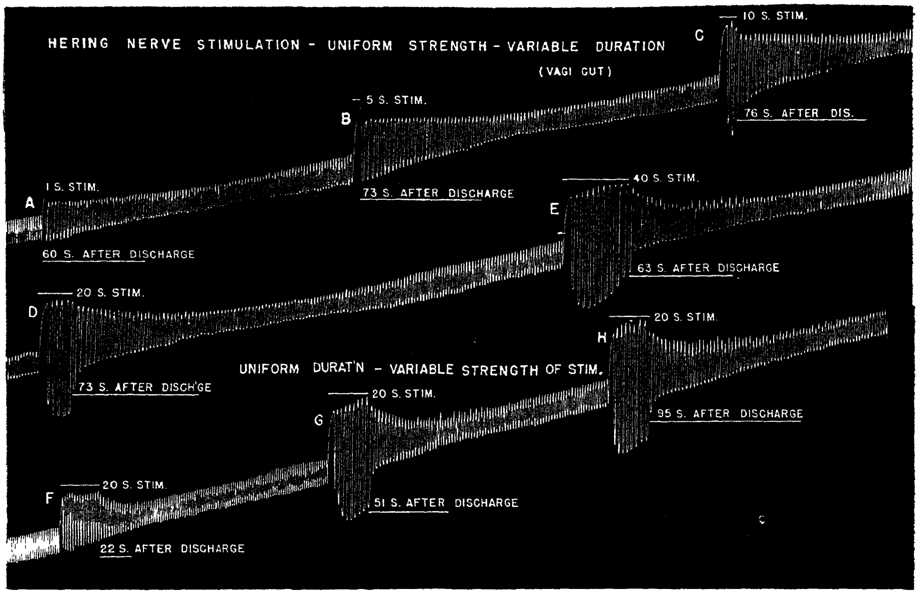


Fig. 3. A, B, C, D and E. Effects of increasing the duration of faradic stimulation of Hering's nerve on the duration of the after-discharge. Chest intact. Comparing A, B and C, increased duration of stimulation is seen to prolong the after-discharge. Comparing C, D and E, a further increase of duration of stimulation is seen to shorten the after-discharge. See text for explanation. F, G and H show effects of increasing strength of stimulation with duration remaining constant. (See last section of paper on intensity, frequency and duration of stimulation.)

are just balanced by the weakening effects of a simultaneously increasing anticholinesterase activity resulting from a diminishing cH.

In figures 4A and B Hering's nerve is stimulated 20, 40 and 70 seconds respectively, also with the chest intact, thus allowing varying degrees of overventilation. The corresponding periods of after-discharge following these graded alkalinizations were 78, 70 and 47 seconds. Toward the end of figure 4B the chest was opened on both sides (see the drop in the spirometer record). Artificial ventilation was immediately administered (see the slower and greater excursions of the spirometer). The stroke of the pump was set as accurately as is possible with a single adjustment to approximate the volume of normal breath-

ing. The slowly diminishing costal excursions, however, indicate that the artificial ventilation was slightly in excess and that the animal was turning slowly

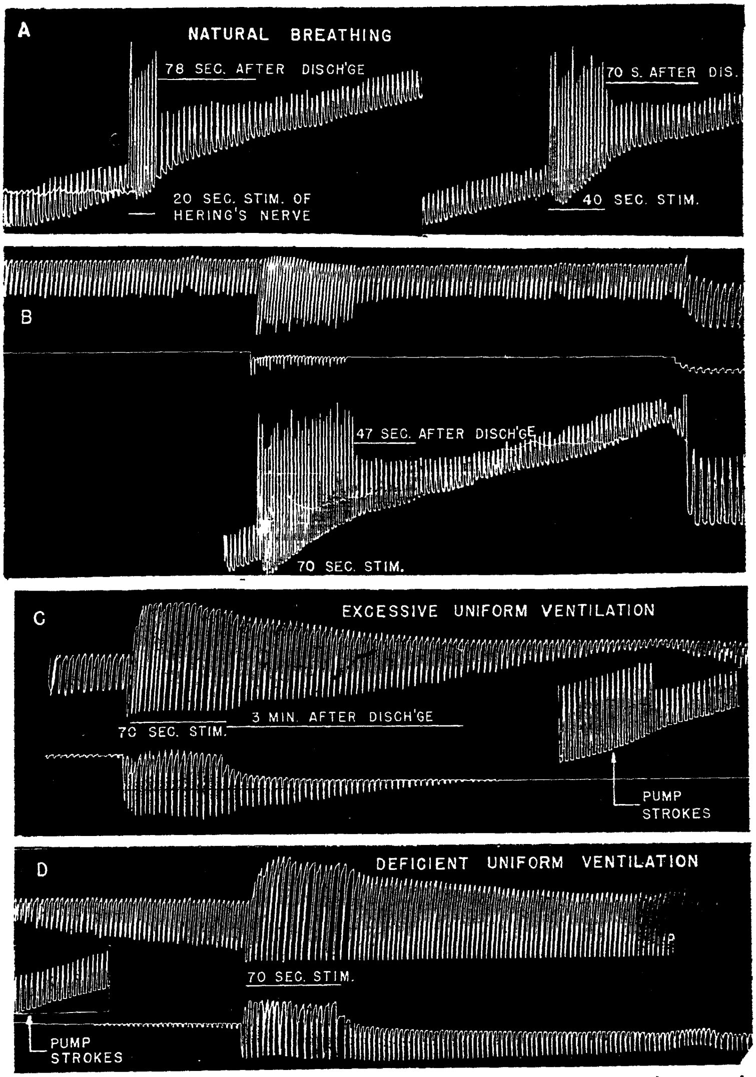


Fig. 4. A comparison of the effects of stimulation of Hering's nerve for a period of 70 seconds under three conditions of acid-base equilibrium. In A and B where the chest is intact and reflexogenic hyperventilation is progressively prolonged the after-discharge lasts 78, 70 and 47 seconds, respectively. In C where the chest is open and artificial ventilation is uniform and only slightly in excess of normal, the after-discharge to stimulation lasts 3 minutes. In D where artificial ventilation is deficient. after-discharge is indeterminably long because the animal is turning progressively more acid.

more alkaline. Under these newly created conditions in which abrupt reflexogenic hypocapnia and *extensive* reduction of cH were eliminated the after-discharge was greatly lengthened—from 47 seconds in figure 4B to 180 seconds in

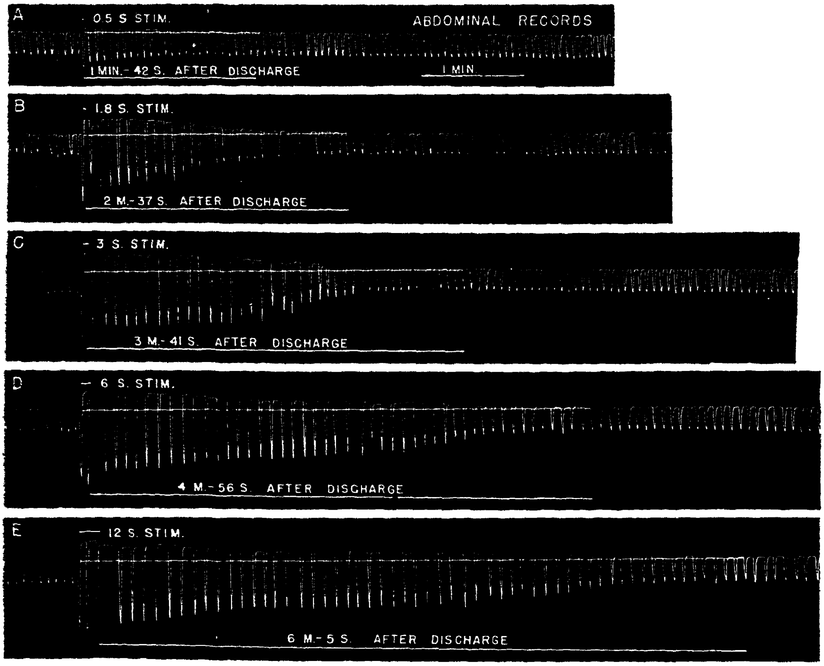


Fig. 5. Effects of duration of stimulation of Hering's nerve during uniform conditions of acid-base equilibrium upon the duration of after-discharge. Open pneumothorax and uniform artificial ventilation adjusted to the basal respiratory requirements. The after-discharge increases in duration with increasing length of stimulation.

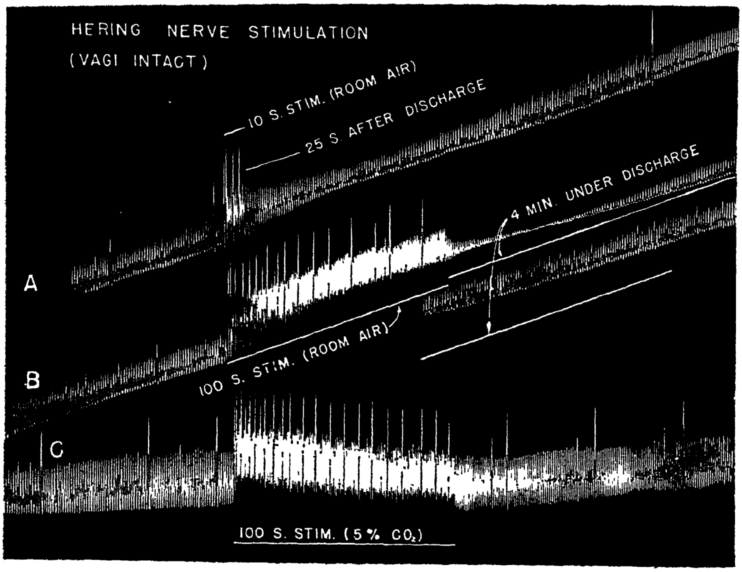


Fig. 6. Comparison of effects of prolonged stimulation of Hering's nerve while the animal breathes room air in B and a carbon dioxide mixture in C. Spirometer tracing. The absence of signs of fatigue under the presumably adverse conditions of C indicates that fatigue does not explain weakening of respiration in either B or A. See text for explanation.

figure 4C—an increase of approximately 400 per cent. In figure 4D where artificial ventilation is deficient as a result of a reduction of the stroke of the pump, recovery from stimulation can not occur for the animal is turning progressively more acid.

In figure 5 the attempt was made to maintain uniform acid-base equilibrium during a series of stimulations of graded duration, thus duplicating the conditions of Sherrington's experiments on spinal animals. The chest was open for a long period under uniform artificial ventilation carefully adjusted to the basal requirements of the animal. Hering's nerve was stimulated for periods of 0.5, 1.8, 3.0, 6.0 and 12.0 seconds respectively. The durations of corresponding after-discharges were 102, 157, 221, 296 and 365 seconds, illustrating an unquestionable similarity of relationship of after-discharge to duration of reflex stimulation in respiratory and spinal reflexes.

Synaptic fatigue and duration of after-discharge. Cannon and Rosenblueth (1937) have presented evidence for the existence of fatigue in motor end plates after very prolonged motor nerve stimulation. They believe that the amount of acetylcholine liberated at each end plate by each impulse is diminished by prolonged stimulation. Therefore it could follow that the amount of acetylcholine in the process of liberation in the respiratory center is less at the end of a long period of stimulation of Hering's nerve than at the end of a shorter period of stimulation. Compare figure 4B where stimulation lasts 70 seconds with that of figure 4A where stimulation lasts only 20 seconds. The shortening of the after-discharge produced by the added 50 seconds of stimulation in figure 4B might then be due to a reduction in rate of liberation of acetylcholine as well as to an augmented speed of its destruction. But when the hypothetical factor of augmented destruction is eliminated by preventing reflexogenic overventilation, as in figure 5, there are no indications that prolongation of stimulation is attended by greater synaptic fatigue. Moreover, in figure 4D where artificial ventilation is reduced below normal requirements the after-discharge is increased above that of either figure 4B or C. Inasmuch as an increase of tissue cH above normal tends to impair oxidations an earlier onset of fatigue might have been expected.

Corroborative findings are illustrated in figures 6B and C in which Hering's nerve is stimulated with equal intensity for periods lasting 100 seconds each. The animal breathes room air in figure 6B and a carbon dioxide mixture (5 per cent CO_2, 30 per cent O_2 and 65 per cent N_2) in figure 6C. Several changes in breathing occur in figure 6B which might be regarded as highly suggestive of fatigue. There is a diminution of hyperpnea during the period of stimulation, a reduction of breathing almost to the point of apnea after stimulation has ceased, and a final and gradual "recovery" to normal. But in the next stimulation, figure 6C, where alkalinization is prevented by the breathing of carbon dioxide and where oxidations are presumably hampered all three of these signs of fatigue disappear almost completely. It therefore seems that synaptic fatigue can play only a relatively insignificant rôle, if any, in the interpretation of our results.

It seems highly improbable that the respiratory center would fatigue in the course of a short reflexogenic hyperpnea lasting only ten seconds, because physio-

logical hyperpneas of great intensities can endure for extremely long intervals of time. If that assumption is correct it is probable that the hypopnea occurring after the termination of stimulation of Hering's nerve in figure 6A is due primarily to the flushing of the tissues of a portion of their normal anticholinesterase activity.

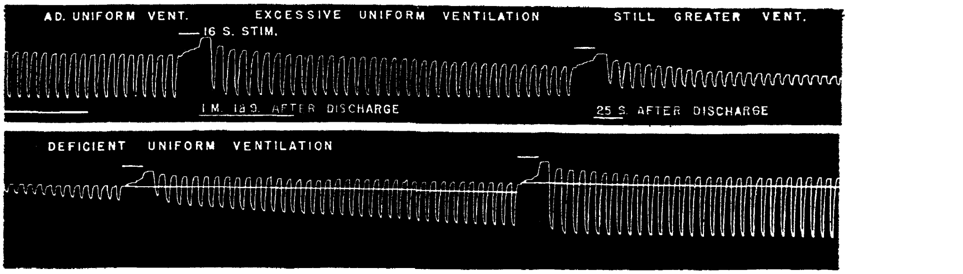
Our findings on reflexogenic hyperpnea are reminiscent of the well-known respiratory response of the dog to intravenous injection of cyanide. If the period of injection is relatively brief the effects produced are very similar to those resulting from electrical stimulation of Hering's nerve—a transitory hyperpnea, an apnea or short period of markedly diminished ventilation and a final gradual return to the normal respiratory volume existing before injection. As is now well-known through the experiments of Heymans, cyanide stimulates breathing by its action on the chemoceptors. The subsequent apnea and hypopnea we believe are due to a temporary alkalinization of the brain which outlasts chemical stimulation at the chemoceptors, because cyanide is rapidly destroyed in the tissues. During this state of super-alkalinity the basal synaptic bombardment of the center is no longer capable of maintaining the normal volume of pulmonary ventilation. Breathing consequently subsides, carbon dioxide necessarily re-accumulates, anticholinesterase activity increases, and pulmonary ventilation returns to normal (Gesell, Lapides and Levin, 1940).

The effects of prolonged stimulation of Hering's nerve are also reminiscent of those produced by voluntary hyperpnea in man. As Haldane has shown, apneas fail to occur if the individual breathes a carbon dioxide mixture instead of pure air and are therefore not due to fatigue.

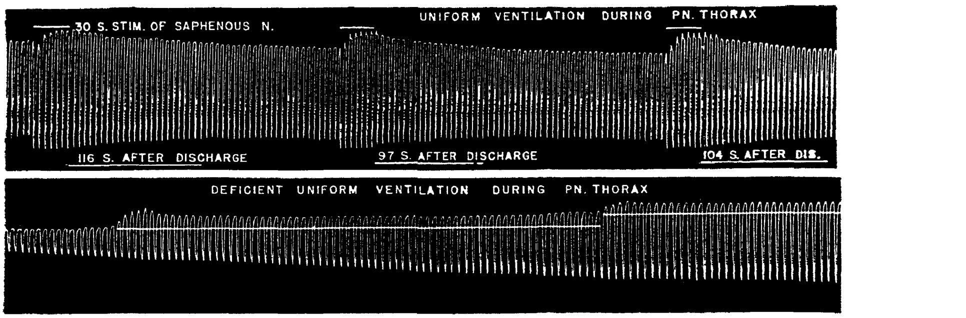
The influence of cH on the reflex respiratory responses to stimulation of the superior laryngeal and saphenous nerves. In contrast to the effect of stimulation of Hering's nerve, stimulation of the superior laryngeal nerve produces a predominantly expiratory response which if sufficiently intense holds breathing in the expiratory phase through reciprocal inhibition of the inspiratory half-center (Gesell and Hamilton, 1941). The excessive expiratory constriction of the chest produced by such stimulation during artificial ventilation is illustrated in figure 7 by the sustained upstroke. Each stimulation lasts 16 seconds. The first stimulation occurs during artificial ventilation just adequate to meet the basal respiratory needs, i.e., the stroke of the pump was so adjusted as to maintain a uniform strength of respiratory contractions as recorded by the costal band tracing. Several minutes preceding the second stimulation the stroke of the pump was increased for the purpose of creating a gradual alkalinization of the animal. It will be seen that expiratory constriction of the chest produced by stimulation is less than before. The stroke of the pump was now reduced to a volume insufficient for basal respiratory requirements and maintenance of a normal cH as is indicated by the continued increasing respiratory movements. In contrast to the effects of alkalinization, acidification increases the degree and duration of the expiratory costal constriction produced by stimulation of the superior laryngeal nerve. As judged by that portion of the record lying above the horizontal extension, the expiratory constriction produced by stimulation of the

superior laryngeal continues indefinitely. This extraordinary prolongation of expiratory after-discharge compares well with the prolongation of inspiratory after-discharge illustrated in figure 1, which indicates that both half-centers share in the acid-humoral mechanism of nerve cell motivation. Such balanced response to changes in cH is no doubt essential to co-ordinated integration of half-centers.

The effects of stimulation of the saphenous nerve differ from those of Hering's nerve and the superior laryngeal nerve in that the inspiratory and expiratory components are more evenly balanced. The balance, however, is by no means



7A



7B

Fig. 7. Acid potentiation of expiratory reflexes elicited by two different nerves; the superior laryngeal in 7A and the saphenous in 7B. Costal band tracings. The results show that acid potentiates expiratory bombardment regardless of the source of the impulses.

perfect and may vary from one experiment to another. In figure 7B, for example, a slight dominance of the expiratory component is evident. In these observations the saphenous nerve is stimulated for equal periods of 30 seconds during uniform artificial ventilation. The ventilation is adjusted to a volume just adequate to meet respiratory needs in the upper record. The after-discharge which consists of a combination of increase of inspiratory and expiratory contractions averages about 105 seconds. Following these observations the stroke of the pump was reduced below the basal requirements of the animal in order to produce a gradual increase of the cH of the tissues. The increasing amplitude of the costal tracings indicates the progress of this change. Under these condi-

tions the expiratory constriction failed to subside with the termination of the stimulation in the manner illustrated in the preceding record, but continued indefinitely. The similarity of the effects of increased cH on the expiratory components of the superior laryngeal and saphenous nerves demonstrates that potentiation of activity of an individual half-center is independent of the source of bombardment of that center.

Intensity, frequency and duration of sensory stimulation. All reflexes should be sensitive to variations in intensity, frequency and duration of stimulation of sensory nerves because the combination of these factors theoretically determines the volume of synaptic bombardment, i.e., the total number of impulses impinging at the centers. In terms of neuro-humoral mediation the effectiveness of stimulation is related to the amount of acetylcholine laid down per unit of time and to the amount concomitantly destroyed. These factors determine the sum total accumulated at the neurones. Strength of stimulation of a nerve trunk decides the number of nerve fibers and the number of synapses activated and therefore the amount of acetylcholine liberated per shock. With strength of stimulation remaining constant, frequency of stimulation fixes the total amount of acetylcholine liberated per unit of time. The time intervening between stimuli determines the amount destroyed between shocks; therefore frequency determines not only the maximum amount which can accumulate but also the rate with which it accumulates, i.e., the gradient of summation of nerve cell motivation. Other factors remaining constant, the duration of stimulation determines how long the process of summation will continue and fixes the amount of acetylcholine which will ultimately accumulate.

The effects of increased duration of stimulation of Hering's nerve have already been noted (fig. 5). Provided hyperventilation is avoided the intensity of the respiratory response increases in conformity with the expected accumulation of acetylcholine. A similar augmentation of response occurs when strength instead of duration of stimulation is increased (see figs. 3F, G and H). When both strength and duration of stimulation are kept uniform the respiratory response also increases with an increase of the frequency of shocks (see figs. 8A, B, C and D). Hering's nerve is stimulated faradically for a period of 60 seconds in each observation, at frequencies of 4, 8, 15 and 30 per second respectively. The effect of shortening the time between shocks is evidenced not only in the response of the main respiratory muscles but in the accessory muscles of the face as well. At the lower frequencies of 4 and 8 per second the accessory muscles remain quiescent. At 15 per second definite respiratory contractions appear and at 30 per second they are markedly augmented. Thus it is believed that the factors of intensity, duration, and frequency of stimulation produce effects upon respiratory reflexes comparable to those known to occur in spinal reflexes.

Fundamental similarities of respiratory and spinal reflexes indicate a common cholinergic integration and a similar modification by cH. The literature abounds in observations on this point, revealing an incidence of acid-humoral mediation wherever acetylcholine is thought to play a physiological rôle. 1. Mathison (1910 and 1911) finds that "Oxygen lack, CO_2 excess, intravenous injection of

acid all cause a rise of blood pressure, and muscular spasms" in the spinal animal and "that the action of carbon dioxide on the skeletal muscle centers is not confined to the respiratory center, but is also capable of affecting the spinal cord; the action differs only in the respect that the medullary center is much more sensitive." 2. King, Garrey and Bryan (1932) conclude that "The lower spinal centers are qualitatively affected by an increase in the carbon dioxide tension in the blood and by anoxia as is the respiratory center, but quantitatively are much less responsive." 3. Montgomery and Luckhardt (1929) show that an "increase in the intraspinal pressure following transection of the cord above the knee jerk center results in a temporary but marked increase in the amplitude of the knee jerk." 4. Schweitzer and Wright (1937) state that the force of the knee jerk is not uncommonly enhanced by ischemia of the cord. 5. Porter, Blair and Bohmfalk (1938) find that slight asphyxia produced by increased intraspinal pressure and intravenous injection of sodium cyanide increases the number of motor units responding to reflex stimulation. 6. Cannon and Haimovici (1939) state that "the simplest way to excite spinal nerve cells is by partial asphyxia." 7. The findings of MacDowall (1930) are more inclusive. "If a decerebrate animal is overventilated the characteristic rigidity disappears and the muscles become quite flaccid but the rigidity returns if CO_2 is caused to accumulate." "The results suggest that if the respiratory centers are considered as the upper ends of the reflex arcs, then the well known stimulating effects of CO_2 on these centers is not specific." 8. Derbyshire, Rempel, Forbes and Lambert (1936) state that a "striking increase in a spinal reflex during asphyxia is similar to that which we have often observed but not measured in decerebrate cats just before the onset of asphyxial convulsions following respiratory failure." "Whereas the flexion reflex was abolished by anaesthetics it was increased in asphyxia." 9. Elsberg and Pike (1926) find that "the susceptibility of cats to convulsive seizures from absinth was increased whenever intracranial pressure was raised by intracranial injections" and "diminished when intracranial pressures were lowered by intravenous injections of hypertonic solutions." They suggest that Jacksonian epilepsy may be due to localized differences in intracranial pressure and that a starvation diet may benefit by a lowering of the intracranial pressure consequent upon the diminished fluid intake. 10. The experiments of Drabkin and Ravdin (1937) disclose the following sequence of events leading to convulsions in hyperinsulinism: severe hypoglycemia, anhydremia, rise in cerebrospinal fluid pressure to critical level, convulsions. They believe that "The previous state of hydration of animals has a profound effect upon the incidence of convulsions in insulin hypoglycemia." Dehydrated animals did not have convulsions. 11. Bartley, Howard and Heinbecker (1940) find that the effect of carbon dioxide (10 per cent) is to produce excitatory effects similar to those produced early in hypoglycemia and by mild strychninization. In this connection it should be recalled that strychnine is an anticholinesterase and therefore should produce effects analogous to those of acidosis during hypercapnia and hypoglycemia. They state that "The excitatory effects following insulin administration are evidenced by a lowering of the threshold, for the

immediate response to stimulation of the saphenous nerve and an increase in its amplitude and duration. This is associated with an intensification and prolongation of the discharge in certain peripheral axons as is illustrated in the efferent discharge over the phrenic nerve." It will be noted that these results coincide with those of ours in which other efferents were stimulated. 12. Salant (1925) calls attention to the stimulation of the vasomotor center in the medulla by nicotine (as reported by Pilcher and Sollmann) and of a similar stimulation by lactic acid as reported by Mathison. Salant finds that the cardio-inhibitory and the pressor action of nicotine are increased by an injection of lactic acid and decreased by a similarly administered alkali. 13. Henderson (1918) showed that overventilation of the lungs lowers blood pressure. 14. Dale and Evans (1922) showed that overventilation produced an increase of leg volume which they attributed to a washing out of carbon dioxide from the medulla. 15. Swale, Vincent and Thompson (1928) showed that the fall in blood pressure is reduced in hyperventilation by the addition of 5 per cent CO_2 to the inspired gaseous mixture. Granting that blood pressure is maintained reflexly in the same manner as is breathing it may be concluded that the intensity of vascular reflexes is graded by the anticholinesterase activity of carbon dioxide in the manner described for the respiratory reflexes. 16. In this connection McDowall (1930) has shown that there is a good vascular reaction to the foot-down position under ordinary experimental conditions but following overventilation this reaction is very poor. 17. Anrep and Segall (1926) state that if cerebral anemia "is produced after section of both vagi only an accelerator effect is observed. This shows that in the first case both centers are being simultaneously stimulated by the anemia, the effect of the inhibitory center predominating over that of the accelerator." 18. Anoxemia was found to increase the cardio-inhibitory action of the vagus by Van Liere and Crisler (1933). 19. In studies on the effects of asphyxia upon the heart rate of rabbits at different ages Bauer (1938) concludes that the gradual appearance of the inhibitory effects of asphyxia is due to the gradual development of vagal action. This confirms our stand that acid does not act as a *direct stimulant.* Its action as a *moderator* is dependent upon the liberation of acetylcholine at the nerve terminals. 20. As shown by Bronk, Tower and Solandt (1935) the postganglionic response becomes progressively larger during the course of repetitive submaximal stimulation of the preganglionic fibers. This presumable facilitation is markedly augmented by restricting the circulation of a perfused ganglion (Bronk and Larrabee, 1937). In a like manner it is found that "the persistent discharges of sympathetic fibers, assumed to be vasoconstrictor, are increased by asphyxia as the blood pressure begins to rise" (Adrian, Bronk and Phillips, 1932). 21. On comparing the effects of prolonged rapid stimulation of the cervical sympathetics on the nictitating membrane, and on the iris, and of similar stimulation of the vagus on the heart, Lanari and Rosenblueth (1939) find that the decline of response during fatigue (4th stage of transmission) is followed by a late persistent increase (5th stage). It is inferred that "the 4th and 5th stages are ganglionic phenomena analogous to the 4th and 5th stages described by Rosenblueth and Luco (1939) for somatic

neuromuscular junctions." It is concluded that "the transition from the 4th to the 5th stage is due to an increase in the ability of the nerve to *produce* acetylcholine" (italics ours). If, however, the cH increases during this period of transition the 5th stage would be more logically explained by the protecting effects of a progressively increasing anticholinesterase activity. The observations of Lanari and Rosenblueth (1939) that unavoidable slight impairment of the circulation in the ganglion becomes a disturbing factor in their studies on the five stages of response would seem to conform with this possible interpretation of the 5th stage. 22. The observations of Babkin (1924) on this point are apropos. He found that "under such apparently very unfavorable conditions (very low blood pressure) the stimulation of the vagi below the heart provoked a very considerable secretion." "Even a few minutes before death when the heart was beating slowly (7 to 8 in 1 min.) and the blood pressure gradually falling to zero, the stimulation of the vagi gave some secretory effect." One of us has had the same surprising experience with salivary secretion elicited by stimulation of the chorda tympani during progressive hemorrhage. At the lower blood pressures salivary secretion was copious enough to extract sufficient fluid from the supply of blood to diminish and in some cases almost obliterate the flow of blood through the gland (Gesell, 1919). 23. The significance of intense activity under unfavorable conditions is further illustrated in the most interesting demonstration by Guttman, Horton and Wilber (1937) of enhancement of muscular contractions occurring after tetanus in the isolated muscle which is being progressively "fatigued" by an infrequent rhythmical stimulation of its motor nerve. Under most unfavorable conditions when the muscle is approaching complete fatigue, indirect tetanization for approximately 10 seconds produces great enhancement. The authors conclude that the accumulation of a chemical mediator at the myoneural junction offers a reasonable explanation of their findings. Joint accumulation of acid and mediator working hand in hand would seem, however, to offer a more complete explanation since the prevention of the formation of lactic acid by iodoacetate modified the results profoundly. The authors state that "Preparations from frogs injected with 20 mgm. of sodium monoiodoacetate showed no enhancement after tetanus given either before or after the onset of fatigue, but instead the tetanus markedly increased the fatigue." 24. Another instance in which cholinergic intermediation is particularly effective under conditions of stress and presumably high cH is illustrated in the experiments of Garrey and Boylsen (1935). "Dinitrophenol augments the respiratory metabolism of resting cardiac muscle in vitro, the rate of oxygen consumption being increased threefold on the average and in some cases as much as fivefold." "Vagus stimulation was still effective under these conditions and there was evidence that the inhibitory mechanism was actually more sensitive than in the normal preparation." 25. It is most interesting that the very highest functions of the brain which have to do with perception and judgment as well as the lowest function in the gut and pancreas are subject to the modifying influence of acid. This is well illustrated in the numerous studies of Gellhorn. The impairment of visual intensity discrimination produced by breathing 8 to 9 per cent oxygen mixtures

is "completely removed or greatly diminished by small concentrations of carbon dioxide" (3 per cent) (Gellhorn, 1936a). 26. "Addition of 3 per cent carbon dioxide to the oxygen nitrogen mixture greatly alleviates or completely offsets the effects of anoxia" on the critical vision fusion frequency (Gellhorn and Hailman, 1943). 27. The excitability of the "auditory and of the visual apparatus is influenced in a similar manner by hyperpnea, O_2 lack and CO_2 excess" (Gellhorn, 1936b). 28. To all appearances the acid-humoral mechanism assumes an important rôle in high altitude physiology. Good effects of ammonium chloride are described on Mount Kamet and in the steel chamber at 347 mm. B.P. as indicated by lower alveolar CO_2, higher alveolar O_2, a lessened degree of cyanosis, a slower pulse rate, and a greater ability to do muscular work (Douglas, Green and Kerzin, 1932). 29. These results on man are supported by Schlitz, Morse and Hastings (1935) on the dog. "Alkalosis induced by the administration of sodium bicarbonate often reduced the capacity of dogs for muscular exercise." "Conversely acidosis following ingestion of ammonium chloride often exerted a favorable effect and in no case appreciably reduced the capacity of the dogs for exercise." "It would appear that acidosis accompanying physical exercise is not to be regarded as a causal factor of fatigue in dogs." 30. Dill and Zamcheck (1940) find that "The addition to inspired air of carbon dioxide renders a given low pO_2 tolerable, not only on account of an increased arterial saturation but because the disturbance of acid-balance is reduced. However, the gain in oxygen saturation is less than results from an equal pO_2, the pCO_2 remaining constant." 31. The experiments of Gibbs, Gibbs and Lennox (1943) are of interest in the support which they give to the participation of the acid-humoral mechanism in the highest functions of the brain. They find that "Eight subjects breathing a mixture of 6 per cent oxygen and 94 per cent nitrogen became greatly confused or unconscious as evidenced by irregular instead of rhythmic tapping, by errors in addition and subtraction, and by failure to remember correctly a list of three common words. In all cases this failure of intellectual function was accompanied by disordered cortical function as manifested by high voltage 2 to 4 per second waves in the electroencephalogram. However, when the mixture was changed to 6 per cent O_2, 5 per cent CO_2 and 89 per cent N_2, all subjects recovered their normal intellectual functions, and the electrical activity of the cortex returned to normal." 32. Since the evidence is now accumulating that acetylcholine plays an important part in the conduction of the impulse in nerve fiber (see Nachmansohn and Fulton, 1943) it will be advisable to look for evidence of a corresponding rôle of the acid-humoral mechanism in conduction as well as in generation of nerve impulses and motivation at the terminal endings. Perhaps the observations of Necheles and Gerard (1930) are pertinent in this respect. They find that "Exposing a stretch of nerve to carbon dioxide causes a marked increase of total action potential, up to five times the initial values. This is true for carbon dioxide concentrations (in oxygen) from 10 to 100 per cent." 33. To all of these observations should be added the statement of Feldberg and Schriever (1936) that "Asphyxia causes acetylcholine to appear in cerebrospinal fluid after eserine and adrenalectomy."

The evidence strongly indicates that an acid moderating mechanism is built into the very foundation of nervous integration.

DISCUSSION. *A comparative analysis of electroclonic and electrotonic theories of intermediation.* The classical electrical theory of so-called "synaptic transmission" of impulses from one neuron to another is essentially an electroclonic mechanism in which time relationships of bombardment are held to play a major rôle. The theory bases its case primarily on the belief that transmission in nerve fiber shows many physiological phenomena common to "synaptic transmission." "Latency, one-way transmission, temporal summation or facilitation, and transmission of the action potential across a non-conducting gap" all of which have been demonstrated on nerve fiber by Erlanger (1939) lead him to put the following hypothetical question—"If an inactive stretch of fiber over 1 mm. in length does not stand in the way of electrical transmission of the impulse is it reasonable to maintain that the discontinuity at the synapse will stop such transmission?" Eccles (1939) states "it may be taken as established that nerve impulses are propagated over the surface of nerve cells exactly as along peripheral nerve fibers." Rushton (1937) and Katz (1937) show that the electrical propagation of a nerve impulse demands a minimal length of excitation before propagation can occur as an all-or-nothing process. The general opinion seems to be (see Fulton, 1943) that "A discharge at a single axon terminal may be insufficient to excite an anterior horn cell." This is but an extension of Rushton's concept that a minimal critical area of excitation is essential for the all-or-nothing detonator response of a nerve cell. Thus, according to Eccles (1939) "detonator summation results from the fusion both of the excited and of the electrotonic areas which spread decrementally from adjacent synapses." Delayed circuits and reverberating circuits (Lorente de No, Ranson and Hinsey, and others) are thought in turn to account for summation and after-discharge common to nerve centers.

From experimental data obtained by anti-dromic stimulation it has been concluded by some observers that cell body as well as nerve fiber conducts impulses both up and down stream. Forbes (1939), however, regards it as an "unproved assumption" that "an antidromic impulse causes a discharge which sweeps through the entire nerve cell." But granting for the purpose of analysis that the nerve cell, like the heart, conducts impulses in all directions, what are the implications?

The first principle of the electrical theory of nerve impulse conduction, as outlined by Lillie (1923) and generally applied to the problem of synaptic transmission, holds that a difference of potential between two points sets up a local circulating current. The effect of this current is to break down the membrane and to initiate an impulse in the region where it emerges from the nerve fiber, the impulses travelling in both directions along the lines of current flow. For simplicity let us picture what may be expected to happen under these hypothetical conditions on a spherical nerve cell provided with a symmetrical arrangement of synapses, each one of which has a critical minimal area of excitation (see fig. 12). In each of these conditions only those boutons facing us need be considered.

1. *Single synaptic discharges.* If only one synapse be discharged (the one at the center of the concentric circles is chosen) an electrical disturbance would radiate in all directions along a circular wave front, due to radiating critical differences of potential. Such a disturbance is comparable to the circular wave set up by dropping a stone into still water. The impulse therefore would spread into the dendrites to the left as well as into the axon hillock at the right. Thus a single synaptic discharge would initiate a nerve impulse in the neuraxon when the radiating wave front approaches the axon hillock.

2. *Symmetrical multiple synaptic discharge.* Three equally spaced concentric circles of synapses in our schema offer a convenient arrangement for considering hypothetical effects of simultaneous discharge of synapses located on the inner and outer circles. Garrey (1914) showed that two contractions started in opposite directions from a common point in a large circle of ventricular muscle canceled out on collision on the opposite side of the circle. Nerve impulses should cancel out in a like manner whenever they happen to meet. Returning to the electrical disturbances of our hypothetical nerve cell, we may picture two summed wave fronts approaching each other; a centrifugal wave front set up by the four synapses of the innermost circle and a centripetal wave front set up by the 13 synapses of the outermost circle. These waves should meet and exterminate each other at the intermediate circle. As a consequence of such cancellation only one synapse out of the total of 17 would be effective toward setting up a nerve impulse, i.e., the strategically located synapse lying at the base of the axon hillock and the head of the neuraxon. The potential difference established by that synapse would be unopposed from the direction of the neuraxon. Consequently current would flow from the area of negativity established by that synapse in the inner circuit, out through the axon hillock, thus initiating a nerve impulse. Therefore, 16 out of the total of 17 synapses will have dissipated their electrical energy to no avail. Compared with the first hypothetical example in which only one synapse was activated, the efficiency is as 6 per cent is to 100 per cent.

3. *Total synaptic discharge.* Or take the extreme situation where all of the synapses covering the nerve cell discharge at precisely the same moment, as is the case when the afferent nerve trunk going to a sympathetic ganglion is stimulated by a maximal shock. Synaptic potentials being equally opposed in all directions preclude the possibility of local circuits excepting at the axon hillock where, as above, current emerges and initiates a nerve impulse. If this reasoning is correct, efficiency of "synaptic transmission" must vary inversely as the number of synapses simultaneously activated and gradation of response to graded stimulation must be impossible.

How then may the problem of nerve cell motivation be approached in conformity with physiological observations? Primarily, we believe, by questioning the rôle of physiological conduction in the soma and predicating a capacity on the part of the nerve cell body to combine individual local electrochemical activities into one common source of current, the intensity and effectiveness of which are determined by the number of synapses simultaneously active, i.e., by

the total area of chemically active surface existing on the membrane. On the basis of these theoretical considerations one may legitimately ask in support of the humoro electrotonic theory, just as Erlanger did in support of the electroclonic theory. If the potential of a nerve fiber suffices to span an inactive gap of approximately one millimeter would not the combined activity of hundreds of synapses be capable of generating sufficient current to stimulate the axon hillock across a comparable inactive cytoplasmic gap between the dendrites and axon hillock? This question assumes particular weight in view of the natural conditions favoring such an electrotonic current (Gesell, 1940a).

It must also be asked how a critical timing mechanism can function in a Mauthner cell motivated by 100,000 impulses per second, arriving over 10,000 synapses via 12 varieties of fibers, each of which may carry impulses at individual frequencies. Now, if it be granted that 5 properly timed impulses suffice to detonate the neuron, in Eccles' sense, the arrival of 100,000 impulses per second would provide conditions for 20,000 detonations per second. Obviously this is above the capacity of response of neurons.

The question then arises—by what mechanism can a nerve cell possibly react effectively to 100,000 stimuli per second in conformance with the electroclonic theory? The neuron might conceivably go into a state of "fibrillation" were it to react like ventricular muscle to frequent stimulation. Assuming such a state of fibrillation to exist in the nerve cell the axon hillock might discharge at a relatively low frequency. The situation would be comparable to that demonstrated by Garrey (1914) in which a small block of ventricular muscle connected by a narrow bridge of muscle with the main fibrillating mass will beat in a rhythmic and co-ordinated manner. But such a mechanism in the nerve cell would be one of chance and not of discrete timing. Moreover it would be one of low efficiency, according to the experiments of Hooker and Kehar (1933) on the fibrillating ventricle.

Many of the major difficulties encountered in the electroclonic theory are escaped in the electrotonic theory. Just as the hydroelectrical engineer simplifies his problem by dividing it into generation, transmission and motivation so may the function of the neuron be divided (see figs. 10, 11 and 12). The generator of the nerve impulse consisting of the cell body and dendrites offers the necessary surface to accommodate the converging bombardment; the synapses ending on the cell body and dendrites liberating the highly electrogenic acetylcholine thus function as electrochemical plates; the choline acetylase-cholinesterase-anticholinesterase combination controls the production and destruction and the amount of surviving acetylcholine; the axon hillock interposes an electrosensitive membrane in the path of the collective electrotonic current generated by the scattered synapses. At this membrane the electrical energy provided by the soma and dendrites is converted into rhythmical electrical discharges or nerve impulses. These impulses are transmitted by the neuraxon to the terminal synapse or end plate where motivation occurs.

One of the difficulties of the electroclonic theory was the contradiction between the alleged slowness of destruction of acetylcholine and the extreme shortness

of the summation interval of electrical stimulation of the nerve cell (Eccles and Lorente de No). Be that as it may, it seemed to us that theory demanded a diametrically opposite stand. We therefore proposed in conformity with our theory of acid-humoro-electrotonic mediation that "Slowness of decay of the electrochemical activity of newly liberated acetylcholine should be an *asset* rather than a *liability* to nervous integration" (Fed. Meetings, 1942). This point of view is explained with the aid of the schema illustrated in figure 9 and figures 10, 11, 12 and 13.

In the monosynaptic cells (Bodian, 1937) possessing but a single large synapse (see fig. 10) it is assumed that sufficient acetylcholine is liberated with a single shock to detonate[3] the axon hillock (see no. 1 of fig. 9). The resulting potential

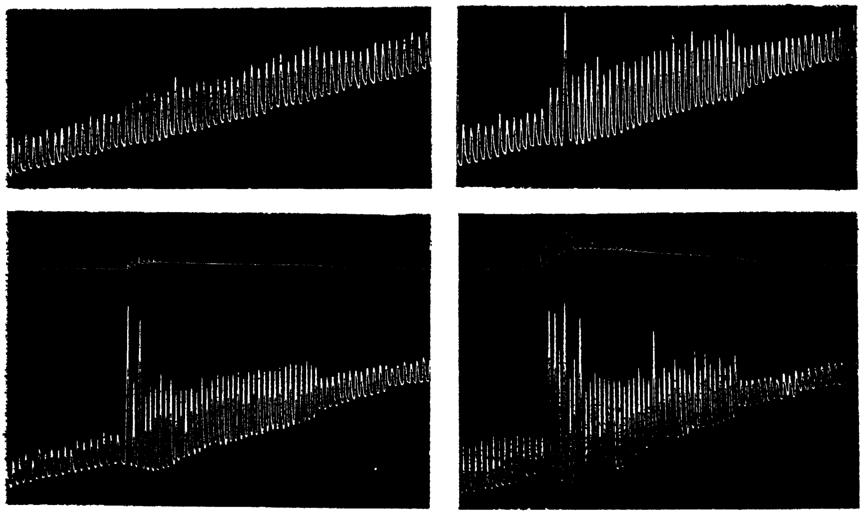


Fig. 8. Effects of frequency of stimulation of Hering's nerve, all other factors remaining constant. Spirometer record of breathing, suspension record of contractions of facial muscles, chest intact, duration of stimulation one minute. Frequencies of stimulation 4, 8, 15 and 30 per second respectively. Note that summation of stimulation was insufficient to activate the facial accessory muscles of respiration at the two lower frequencies of stimulation.

difference between the synapse and the axon hillock detonates the hillock as soon as the threshold of stimulation is crossed (see horizontal line). The potential falls toward subthreshold level as soon as the rate of hydrolysis of acetylcholine overtakes the rate of its liberation. If the E.M.F. recrosses the threshold before the refractory period of the axon hillock has ended only one detonation per synaptic discharge will occur, as illustrated by the axon hillock discharge.

On the other hand, if the frequency of stimulation is increased the acetylcholine is only partially destroyed between stimulations. The E.M.F. consequently remains at a higher level and the axon hillock detonates rhythmically at a higher

[3] Detonation is assumed to be confined to the axon hillock, not to include the cell body.

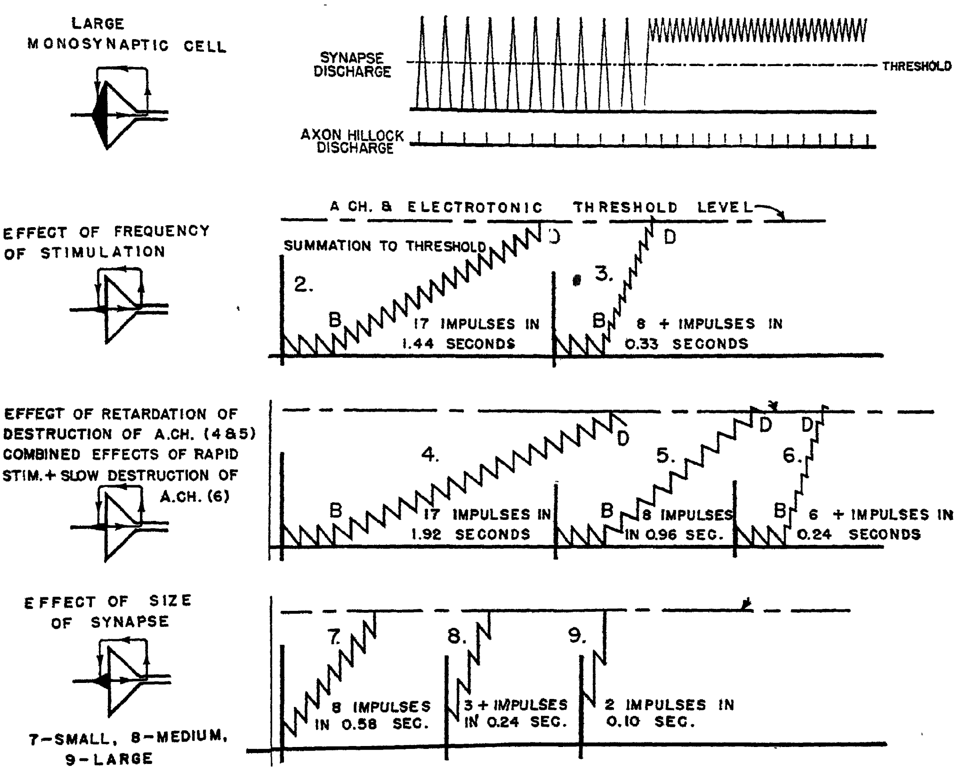


Fig. 9. A schematic representation of the electrotonic mechanism of summation of synaptic potentials. The amount of free electrogenic acetylcholine and the corresponding synaptic potential obtaining with each synaptic discharge is plotted on the ordinates as a common curve against time on the abscissae. The threshold level of the electrotonic current producing detonation of the axon hillock is represented by a horizontal line above the abscissae. No. 1 represents the theoretical effects of infrequent and subsequent more frequent discharge of the large synapse of a monosynaptic cell. Comparison of the frequency of axon hillock discharge with the frequency of synaptic discharge shows that they correspond only with the low frequency of synaptic discharge. Nos. 2 to 9 show the effects of repetitive discharges of a synapse too small to detonate the axon hillock without the aid of temporal summation. Nos. 2 and 3 show the effects of frequency of synaptic discharges. When the rhythm of discharge is low the acetylcholine which is liberated with each discharge falls to the zero line between discharges. At 2B where the rhythm is increased each synaptic discharge leaves an acetylcholine residuum. These slowly accumulate during the course of 17 synaptic discharges up to the threshold level where the axon hillock is detonated. At 3B a higher frequency of discharge leaves larger residua per discharge and threshold level is reached in 8 discharges. The combination of increasing residua and frequency of synaptic discharge shortens the latent period of detonation of the axon hillock to approximately 25 per cent of that of 2B. Nos. 4 and 5 show the effects of retardation of hydrolysis of acetylcholine, frequency of stimulation remaining constant. Moderately increased anticholinesterase activity introduced at 4B leaves acetylcholine residua which reach threshold level in 17 impulses. Stronger anticholinesterase activity introduced at 5B leaves larger residua. The axon hillock is discharged by 8 instead of 17 impulses. A combination of increased frequency of impulses and anticholinesterase activity in 6 leads to more rapid summation and a greatly decreased latent period of axon hillock detonation. Nos. 7, 8 and 9 show the effects of size of synapse, frequency of impulses and anticholinesterase activity remaining constant. The larger the synapse the greater the acetylcholine residua between impulses and the steeper the acetylcholine gradient and the shorter the latent period of axon hillock detonation. Detonation is reached in 9, 5 and 3 impulses with the small, medium and large synapses respectively. (Exhibited as a demonstration at the meetings of the Federation of American Societies for Experimental Biology, 1942.)

frequency which is related to the intensity of the electrotonic current so generated. As illustrated in our schema the frequency of response is out of phase with the frequency of stimulation.

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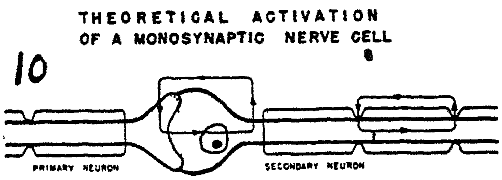

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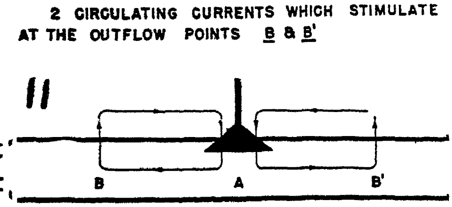

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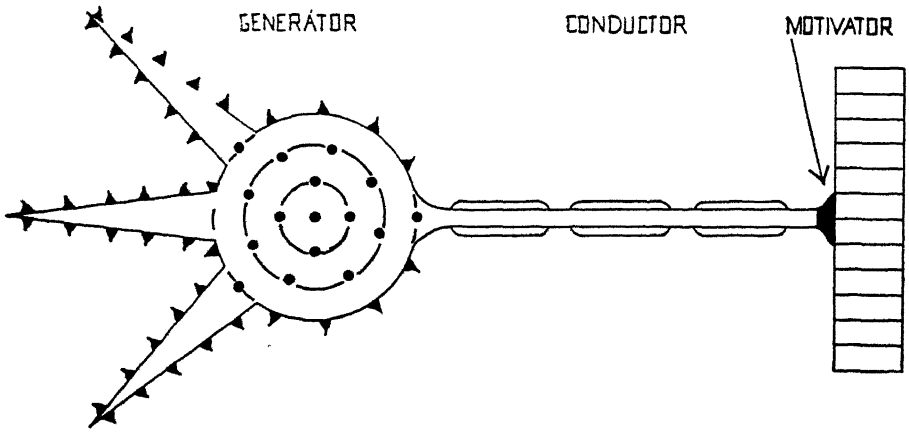

Fig. 10. The electrical circuit of a monosynaptic nerve cell as postulated by the electrotonic theory of nerve cell motivation. The nerve impulses are generated by the stationary electrical circuit shown emerging at the axon hillock. They are propagated by the advancing electrical circuit shown emerging at the internodes, according to the theory of Lillie.

Fig. 11. The double electrical circuit of a monosynaptic muscle fiber as postulated by the electrotonic theory of motor end plate stimulation of muscle (Gesell, Mason and Brassfield, 1944). A continuing electrotonic current set up by exogenous acetylcholine at the end plates (see figs. 1D and E) sets up a prolonged tetanic contraction. On removal of the muscle from the acetylcholine environment the muscle continues to contract with weakening intensity comparable to the after-discharge of nerve cells to synaptic bombardment.

Fig. 12. A schematic symmetrical nerve cell illustrating the three functional divisions of a neuron: the generator which includes the cell body, dendrites, synapses and axon hillock; the conductor or neuraxon extending between the axon hillock and terminal plate; and the motivator or the terminal plate ending either on a nerve cell or end organ proper. The synapses are arranged concentrically to permit an analysis of the pros and cons of the electroclonic and electrotonic theories of nerve cell function (see text for explanation).

In a multi-synaptic nerve cell in which individual synapses are small, detonation theoretically occurs only through the intervention of either temporal or spatial summation or of both. The most obvious factors affecting the degree

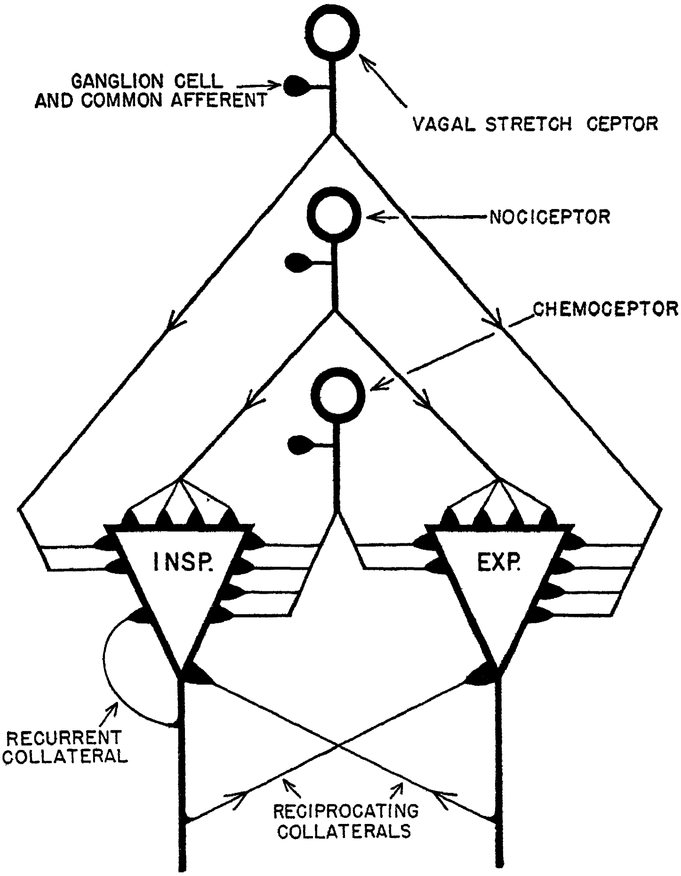

Fig. 13. Schema showing the arrangement of synapses as postulated in the concepts of dual excitatory stimulation of sensory afferents and reciprocal inhibition. Respiratory proprioceptive afferents of the vagus and superior laryngeal nerves have a larger number of terminal synapses ending on the neurons of the expiratory half-center. Chemoceptor afferents have a larger termination on the neurons of the inspiratory half-center. Nociceptors of sensory cutaneous nerves have a more balanced termination. These three groups of afferents impinge on the excitatory poles of both inspiratory and expiratory neurons. The reciprocating collaterals impinge on the inhibitory poles of both inspiratory and expiratory neurons. Afferent bombardment of the excitatory poles by the proprio, chemo and noci-ceptors increases the stimulating electrotonic current and bombardment of the inhibitory poles by the reciprocating collaterals opposes and therefore diminishes it. The afferent fibers tend to drive both half-centers simultaneously. Co-ordinated rhythmic breathing, however, results from the rhythmic alternating activity of the expiratory and inspiratory reciprocating collaterals which alternately release and oppose the electrotonic currents in the inspiratory and expiratory neurons. When exogenous acetylcholine is injected into the vertebral artery it reaches all synapses, excitatory and inhibitory. Both excitation and inhibition are intensified and breathing remains co-ordinated.

of temporal summation are frequency of synaptic bombardment, speed of destruction of liberated acetylcholine and size of the synapse (see fig. 9). Nos. 2 and 3 illustrate the influence of frequency of stimulation, nos. 4, 5 and 6 the

influence of the speed of destruction, and nos. 7, 8 and 9 the influence of the area of the synapse.

Liberation and destruction of acetylcholine and the corresponding changes in E.M.F. are represented by rising and falling gradients respectively for each synaptic discharge. For simplicity of analysis the speed of liberation of free acetylcholine is assumed to be instantaneous, and is so indicated by a vertically rising gradient.[4] The speed of destruction of acetylcholine is a highly variable factor and is represented by its down gradient as a slower process.

When stimulation of a nerve cell through a synapse is infrequent sufficient time elapses between synaptic discharges to allow complete destruction of the liberated acetylcholine. Such conditions are represented in the three initial discharges of no. 2. When, however, the frequency of discharge is increased at *B* the time between discharges is no longer sufficient to allow complete destruction, and one residuum of acetylcholine adds to another over the prolonged summed gradient The electrotonic current rises in steps until it crosses the threshold level when detonation of the axon hillock is assumed to occur. If now the frequency of synaptic discharge is still further increased, as shown in no. 3, the individual residua of acetylcholine become greater, the steps become steeper and the threshold is crossed in the course of 8 discharges, as compared with 17 in no. 2. This, we believe, is the basic mechanism explaining the greater effectiveness of frequent stimulation common to spinal and respiratory reflexes illustrated above in figure 8.

A retardation of hydrolysis of free acetylcholine through increased anticholinesterase activity should theoretically have the same effects on stimulation as does an increase of frequency of synaptic bombardment. The mechanism by which this result is believed to be attained is schematized in no. 4. A relatively small retardation of hydrolysis of acetylcholine following each synaptic discharge is represented by a diminished down slope beginning at *B* where carbon dioxide or eserine is assumed to be administered. Acetylcholine residua build up to threshold value as noted above with increasing frequency of stimulation. In no. 5 where the anticholinesterase activity is assumed to be greater than in no. 4 the detonation threshold of acetylcholine is reached within a shorter period of time, i.e., within 8 synaptic discharges as compared with 17 in no. 4. Obviously a combination of increased frequency of synaptic bombardment plus increased anticholinesterase activity should be more effective than either alone (see no. 6).

As implied above, the amount of acetylcholine liberated and the amount of electrotonic current generated varies with the area of the synapse. Thus the acetylcholine residuum obtaining at a given moment after synaptic discharge must increase with the size of the synapse and thereby greatly modify the speed of summation. The effects of synaptic area are shown in nos. 7, 8 and 9. In no. 7 where the area is small and summation slow, detonation of the axon hillock is shown to occur within 8 synaptic discharges; in no. 8 where the area is of

[4] It should be remembered in this connection, however, that Brown and Eccles (1934) have shown that acetylcholine liberated by vagal inhibition of the heart begins to be destroyed as soon as it is liberated.

medium size, detonation is seen to occur within 3+ synaptic discharges; in no. 9 where the area is still greater only two synaptic discharges are required to detonate the axon hillock.

Our emphasis on the rôle of synaptic area, an anatomical and physiological extension of the views of Lillie, is beautifully supported by the drawings of Bodian showing basket-like systems of 10 or more knob and club-like endings all derived from a single large axon arranged about an ocular motoneuron. Surely each synaptic discharge in such a group of endings must summate simultaneously and be equivalent to the effects of a discharge of a correspondingly large monosynaptic ending. At least it is difficult to see how this arrangement accommodates itself to synaptic scales and to impulse conduction by the soma as required by the electroclonic theory.

It is highly improbable that even repetitive activity of single synapses in neurons abundantly provided with synapses (anterior horn cells and Mauthner's cell, for example) could detonate the axon hillock. In accordance with the electrotonic theory local subliminal potentials become effective only through the process of spatial summations of scattered subliminal summations of individual synapses, since the theory presupposes that electrochemical activities, regardless of their area of scattering, add their quotas to the common excitatory current. The electroclonic theory, on the other hand, has denied summation of impulses arriving at separate dendrites; only those impulses properly grouped as to time and location become effective (Eccles and Lorente de No).

The concept of temporal and spatial summation of scattered asynchronous electrical activities into an electrotonic current have been helpful in our hands in explaining many neurophysiological observations (Gesell, 1939, 1940a, b, c; Gesell, Lapides and Levin, 1940; Gesell, Atkinson and Brown, 1941; Gesell and Moyer, 1941; 1942; Gesell and Worzniak, 1941; Gesell and Hamilton, 1941; Gesell, 1941; Gesell, Brassfield and Hamilton, 1941, 1942; Gesell, Brassfield, Hansen and Mason, 1942; Gesell, Moyer and McKittrick, 1942; Gesell, Hansen and Worzniak, 1943; Gesell and Hansen, 1943; Gesell and Atkinson, 1943). The validity of our interpretations has been strongly supported by the recent direct observations of Eccles (1943) on synaptic potentials of the stellate ganglion in which "synaptic transmission" is blocked or partially blocked by curare. He places one electrode at the pole of the ganglion and the other at the cut ends of the cardiac nerves. In our opinion this arrangement registers the electrotonic current described in the communications of Gesell (1939, 1940a).

Eccles (1943) finds "With repetitive stimulation at very low ratios (5 sec. or less) each successive synaptic potential is practically independent of the preceding. It is superimposed upon a negligible residuum of the preceding potentials and its size and time course differ but little from the initial single response." ... "With moderate rates of stimulation (28 per sec.) there is a stepped rise to a wavy plateau, which is well maintained even during several seconds' stimulation. The plateau is attained earlier and is higher, the faster the stimulus frequency." ... "With still higher rates of stimulation (84 to 140 per sec.) the plateau is still higher and shows little or no sign of undulations corresponding to the stimulus

frequency." ... "When the summated synaptic potential resulting from presynaptic stimulation is sufficiently large, after-discharges occur, and these are prolonged and intensified by the use of physostigmine" (Eccles, in press, cited by Lloyd, 1944). As a result of these recent experiments Eccles now abandons the so-called "detonator response" and concludes, "With curarized ganglia there would appear to be no doubt that the synaptic potential is entirely responsible for synaptic transmission that occurs on account of the summation of successive volleys." A close comparison of results and figures of Eccles with our schema and our experimental results will show a most remarkable correspondence.

It should be remembered that artificial stimulation as employed by Eccles leads to synchronized volleys of bombardment which accounts for the step-like rise of the synaptic potential. In the experiments of Barron and Matthews (1938) slow potentials of the ventral roots were found to increase progressively in a non-step-like fashion during a gradually increasing pressure on the toes. This, no doubt, is due to the fact that the bombardment which such stimulation produces is of an asynchronous nature. Barron and Matthews find that these "slow potentials in the ventral roots always precede any discharge of impulses and the frequency of impulse is related to the level and rise of the potential." This accords perfectly with our concept of electrotonic motivation of nerve cells.

Added background for electrotonic motivation of nerve cells is found in the rhythmic response of other physiological structures to uniform conditions—the rhythmic response of Nitella to a droplet of chloroform placed on its membrane (Osterhout and Smith); the rhythmic response of cardiac muscle to a uniform flow of current; the closing tetanus of Pflüger; the rhythmic response of nerve fiber to its current of injury (Adrian); and the rhythmic activity of sensory receptors (Adrian).

Inhibition. There are reasons for believing that the respiratory center is driven by several varieties of dual excitatory afferents each of which bombards the inspiratory and expiratory half-centers simultaneously (Gesell, 1940a). The proportion of the drives which they provide is thought to vary with the type of sensory fibers, as schematized in figure 13 for the chemoceptive, sensory cutaneous and vagal afferents. The relative dominance of the inspiratory bombardment by chemoceptive afferents is seen in the prolongation of the inspiratory contraction and the absence of after-reinforcement of the expiratory contraction produced by electrical stimulation of Hering's nerve in figure 1A (see also figs. 6A and 8). (Strong stimulation, however, adds appreciably to the expiratory component as is seen in fig. 3.) The powerful dominance of expiratory bombardment is seen in the sustained expiratory contraction produced by electrical stimulation of the superior laryngeal nerve in figure 7; the vagus nerve produces similar effects.

Balanced heterogeneous bombardment, such as occurs under physiological conditions, even though driving both half-centers simultaneously results in an alternating co-ordinated activity due to an alternating inhibition. When an imbalance of normal bombardment is created by stimulation of predominantly

inspiratory or expiratory afferents, either half-center may be held in excessive abeyance. In figure 1A, for example, the expiratory half-center is held in abeyance during the entire period of stimulation of Hering's nerve, and in figure 7 the inspiratory half-center is inhibited during the period of stimulation of the superior laryngeal nerve.

It has been held by many authors that vagal stimulation checks the inspiratory act by a direct reflex inhibition. In a like manner it could be reasoned that the inhibition of the expiratory act occurring during the stimulation of Hering's nerve in figure 1A is also produced by a direct reflex inhibition. Such a stand, however, seems untenable, not that the existence of direct inhibition is denied, because undoubted reflex inhibition does occur when the carotid sinus afferents are stimulated by inflation of the sinus. The evidence for this conclusion lies in the fact that both inspiratory and expiratory contractions are simultaneously weakened. Since excessive inhibition of reciprocal origin demands concurrent excessive activity of the opposing half-centers, reciprocal inhibition is excluded. It would, however, be unjustified to draw the inference that direct inhibition is a common physiological occurrence. Unfortunately most of the inhibition which has been studied in spinal animals is associated with a simultaneous increase of nervous activity in some portion of the cord, e.g., when a contralateral flexion reflex is inhibited by a homolateral reflex. This inhibition could represent a coupled reciprocal inhibition.

The phenomenon of inhibition is more readily studied in the nervous control of breathing where the specialization of sensory nerves comes to our aid. Experiments in this field have brought us to the opinion that the inhibition of inspiration produced by stimulation of the vagus nerve is a reciprocal process between half-centers resulting from excessive excitation of the expiratory half-center. The inhibition of expiration occurring during stimulation of Hering's nerve may be regarded as a similar process. The reciprocal nature of inhibition is supported by the co-ordinated breathing produced by a crude shapeless chemical stimulation of the center, resulting from injection of acetylcholine into the vertebral artery. Co-ordinated breathing can occur only through the mediation of a co-ordinated inhibition. Since such an injection possesses no power of selective alternate inhibition of half-centers the co-ordinated breathing which occurs strongly implies the importance of reciprocating inhibitory fibers in the central architecture of half-centers. It is this reciprocating mechanism which is thought to switch a continuing simultaneous bilateral drive from one half-center to another. Furthermore these observations tend to minimize the importance of direct reflex inhibition originating in peripheral receptors, a view in full accord with Weiss' (1941) concept of automaticity of motor centers.

One point seems reasonably certain—both stimulation and inhibition should be either electroclonic or electrotonic in nature. We, therefore, believe that the preponderance of evidence in favor of electrotonic motivation precludes the probability of electroclonic mechanisms of inhibition.

In the theory of inhibition suggested by Barron and Matthews (1938) nerve impulses are assumed to suffer block produced by slow potential changes elec-

trotonically transmitted in the cord. This theory is criticized by Eccles (1939) on the improbability of chance anatomical relationships producing a co-ordinated inhibition.

Marrazzi's (1939) theory of dual humoral mediation suggests that one humor stimulates and the other inhibits. This chemical neutralizing mechanism has been objected to by Fulton (1943) on the score that no humoral inhibitory "agent has been found; nor is there any evidence for two kinds of fibers, the excitatory and inhibitory, nor for two types of endings of one type of fiber." We, on the other hand, would be inclined to question the theory on the score of economy of energy and performance and the fact that a single humor (acetylcholine) injected into the respiratory center produces highly co-ordinated rhythmical contractions involving both activation and inhibition. The theoretical advantage of mediation by a single humor is that the motivating power persists in both half-centers and is continually available, like steam pressure or E.M.F., ready to be switched at any moment to the axon hillock of either inspiratory or expiratory cells. On the other hand, if two humors were employed, one activating and the other inhibiting, time and energy would be required to rebuild the two processes, activation and inhibition, from zero level with each periodic cycle of each half-center.

In our electrotonic mechanism activation and inhibition are viewed as basically similar phenomena—activation being associated with an increasing and inhibition with a decreasing intensity of the electrotonic current. Thus identically similar synapses when strategically placed in the proximity of the axon hillock instead of at the dendrites must theoretically work to create a reduction of the electrotonic current, i.e., inhibition (see fig. 13). This mechanism differs from that of Barron and Matthews (1938) in that it controls the *generation* and not the *conduction* of impulses. It is the only theory of inhibition, so far as we know, supported by anatomical evidence. In fact it had its origin in the striking arrangement of the synapses and nerve fibers about the axon hillock of Mauthner's cell. It was this peculiar structure that suggested to the neurohistologists that the dendritic and neuraxon ends of the nerve cell have opposing functions. To the physiologists and physicists the arrangement of the boutons in Mauthner's cell have the earmarks of a simple arrangement for adjusting potential drop which may well demand attention in an otherwise barren outlook in the solution of the nature of inhibition.

The relation of acid-humoral mediation to the problem of inhibition is most pertinent. Granting that central nervous integration is a monohumoral phenomenon and that acetylcholine mediates inhibition as well as activation of nerve cells, it follows that both processes would be intensified by an increase of free acetylcholine at the activating and inhibiting poles of the neurons. This seems to hold for an increased liberation of endogenous acetylcholine produced by stimulation of Hering's nerve and for administration of exogenous acetylcholine via the vertebral artery. Therefore an increased accumulation of acetylcholine resulting from the anticholinesterase activity of carbon dioxide should also intensify both processes of activation and inhibition as it seems to do in a

coordinated way in hypercapnic hyperpnea. There is no contradiction in the equal potentiation of activation and inhibition by carbon dioxide since these processes should alternate in perfect co-ordination. In fact the theory of integration of half-centers would seem to demand a double potentiation.

The evolution of humoral intermediation. The exceptional correlation of the cholinesterase content and the voltage delivered by the electrical organ of fishes (Nachmansohn, Cox, Coates and Machado, 1942) seems almost prima facie evidence for cholinergic integration where acetylcholine and cholinesterase are found to occur. The gastropods and cephalopods thus register the use of cholinergic mechanisms for hundreds of millions of years. While the stage at which humoral mediation appears in the evolutionary scale must be regarded as uncertain, an enquiry into the circumstances leading to its development is nevertheless of greatest interest.

The absolute need of free energy is without doubt the first requirement of life. Of the many factors evolved to meet these requirements two in particular are pertinent to our problem: 1, the increase of cH which results from either an increase of activity or decrease of supply of oxygen; 2, the potentiating action of cH on the integrating function of acetylcholine. It is conceivable that properly combined these two factors might force the organism to automatically seek the necessary energy for life.

Respiration thus assumes a most significant rôle in the development of nervous integration. In such forms as the squid, motor activity and breathing are inextricably knit together. Locomotion is increased only with an accompanying increase of breathing since the water which propels the animal is jetted past the gills as it escapes the mantle cavity. Much the same arrangement exists in the mackerel which is without the power of opercular respiration (Krogh, 1940). This fish swims and breathes with its tail and is by this contingency forced to move incessantly through the water. It pursues its oxygen as it pursues its prey. The faster its prey, the greater is its supply of oxygen as the water rushes with increasing volume past its gills.

In fishes so constructed intimate intermeshing of movement and breathing presumably demand equal sensitivity of the upper and lower levels of the central grey axis stem to cH. But in those fishes which employ opercular breathing and are capable of meeting their respiratory requirements without locomotion, the need for this equal sensitivity no longer exists; in fact it might actually work to a disadvantage. The differences in sensitivity of higher and lower levels of the mammalian central nervous system established by numerous authors may, therefore, be looked upon as an evolutionary adaptation avoiding a general excessive motor activity during respiratory stress when actually it is only the augmented breathing which the emergency demands. It is interesting to enquire whether the differences in concentration of carbonic anhydrase in the central nervous system (Ashby, 1944) are in any way related to the differences in sensitivity to cH.

The great asset of humoral mediation is its adaptability to evolutionary requirements. Summation and after-discharge, the key phenomena of cholinergic

terminals, are correspondingly developed in varying degrees in the central nervous system, in striated and smooth muscle, in the heart and secretory glands. Where summation and after-discharge serve to advantage they are found to be accentuated and where they would tend to interfere they are found to be repressed.

Obviously a marked extension of after-discharge at the motor end plates of rhythmically contracting respiratory and locomotor muscles would seriously interfere with refined co-ordination and efficient use of mechanical energy by promoting simultaneous contractions in opposing muscles. Prolonged after-discharge in the gut, however, is not beset with the same dangers.

On the other hand, after-discharge becomes the keystone of integration in the centers, through the mechanisms of reciprocal inhibition as set forth above and in figure 13 (see also Gesell, 1940a).

Still another but entirely different type of adaptation of humoral mediation is seen in the extremely high voltages generated by the electrical fishes. Here the terminal end plates of the electrical organs are enlarged and arranged in series. Tremendous amounts of acetylcholine are alternately liberated and broken down. High concentrations of cholinesterase accomplish the latter (Nachmansohn, Cox, Coates and Machado, 1942) and high concentrations of acetylcholin-acetylase newly discovered by Nachmansohn and Machado (1943) might possibly accomplish the former.

The application of cholinergic physiology to aviation. Man has never been consistently subjected to prolonged rigors of oxygen stress. In choosing to live in the comforts of oxygen plenty he has denied evolutionary forces and is now paying the price of maladjustment to high altitudes in the modern biological requirements of combative and commercial aviation. The chemoceptors, inherited from the fishes, give evidence of rendering valuable service at low or uniform altitudes, but at high altitudes or rapid ascents they play havoc with the body as a whole. Strange as it may seem, their extreme sensitivity to oxygen and the hyperventilation which they produce appear to be the cause of man's maladjustment. While they function to maintain the needed supply of oxygen they rob the body of its physiological stores of acid. The ground for this belief is firm. Even an anesthetized dog, whose nervous integration is relatively insensitive, when subjected to a 7 per cent oxygen mixture not only maintains his usual rate of oxidation but increases it considerably above normal (Gesell, Krueger, Nicholson, Brassfield and Pelecovich, 1932). Hyperventilation sweeps out the carbon dioxide in great amounts as witnessed by the marked increase of the "expiratory quotient." Computations show clearly that the body as a whole has turned more alkaline. Studies on respiratory reflexes yield similar conclusions (Gesell, Lapides and Levin, 1940). Under ordinary circumstances removal of chemoceptor support by bilateral Hering's nerve block after bilateral vagotomy produces only a slight reduction in pulmonary ventilation. The same procedure during hypo-oxic hyperpnea, however, may stop breathing completely. With decreased anticholinesterase activity, i.e., low cH extra chemoceptor

bombardment alone is no longer adequate to drive the respiratory center. Like the individual who forcibly overventilates his lungs and robs his tissues of anticholinesterase activity and swoons with dizziness, the aviator who automatically controls his breathing at high altitudes becomes confused and mentally inefficient. The marked reduction of the acetylcholine content of the brain resulting from hypo-oxia (Welsh, 1943) may be of significance in this respect.

Evidence points to a cholinergic imbalance. This view rests heavily upon the important concepts of von Euler, Liljestrand and Zotterman (1941) on the nature of the carotid bodies. These ganglionic structures, highly sensitive to nicotine and acetylcholine seem to act as cholinergic outposts of the central nervous system for the control of breathing. The convincing evidence of Bernthal and of Winder that cH is the normal moderator of chemoceptor activity during hypercapnia, ischemia, hypo-oxia, cyanide poisoning and hyperthermia provide the second requirement for the concept of cholinergic imbalance (see Bernthal's review, 1944).

What practical approach to aviation does this concept suggest? Beneficial effects of administration of acid during hypo-oxia were enumerated above. As suggested by several authors the improvements are not confined solely to the increased supply of oxygen entailed in the correction of the acid-base equilibrium. The salutary effects resulting from this correction are due, we believe, to the improvement of acid-humoral intermediation in the central nervous system. Judging by the surprisingly high tolerance of tissue oxidations to cH changes (Canzanelli, Greenblatt, Rogers and Rapport, 1939) the relatively minor suppression of oxidations produced by an increase of cH is probably outweighed by the accompanying improvements of nervous integration.

Physostigmine, prostigmine and other anticholinesterases might possibly insure a normal brain efficiency at high altitudes in several ways: 1, by virtue of their direct anticholinesterase activity they might counteract the effects of the lowering of cH on the brain; 2, by virtue of the increase of cH which physostigmine produces (Brassfield, Hansen and Gesell, unpublished results) they would counteract the expected acid-base disturbance per se; 3, by virtue of the increased breathing they produce they would tend to correct extreme oxygen shortages.[5] It is also conceivable that a studied diet might be helpful. Addition of sugars to perfusing solutions is known to increase the production of acetylcholine and to improve the function of ganglion cells (Kahlson and MacIntosh, 1939). The invigorating effects of candy on mental as well as physical exercise are common experience. Potentiating effects of adrenalin on cholinergic mediation have been described by Bulbring and Burn (1942–43). Perhaps the administration of choline acetylase might foster the synthesis of acetylcholine, particularly if the building stones of acetylcholine were made more available. The application of

[5] It should be borne in mind, however, that a central imbalance resulting from the administration of physostigmine in which one half-center becomes more active than the other could embarrass breathing and thereby do harm instead of good (see Gesell and Hansen, 1943).

cholinergic physiology to the problems of aviation may possibly lead to gratifying results not only in protecting individuals at high altitudes but in raising the ceiling of aviation as well.

Hypertension and other nervous disorders. Four general corrective measures call for attention in all forms of cholinergic disturbances: 1, physiological adjustment of the liberation of acetylcholine by controlling the volume of synaptic bombardment; 2, chemical adjustment of the amount of acetylcholine liberated by individual synaptic discharges by use of choline acetylase and by control of availability of sugars, etc.; 3, adjustment of the rate of hydrolysis of acetylcholine by control of the acid-base equilibrium by administration of cholinesterase and anticholinesterases and by control of the amount of acid formed; 4, adjustment of excitability of the axon hillock to prevailing cholinergic electrotonic currents by sedatives, atropin, etc.

Since certain aspects of hypertension may be the result of over-bombardment and over-accumulation of acetylcholine at the vasoconstrictor center its correction would differ from that of high altitude disturbances. Tentative procedures might include: 1, a reduction of physiological bombardment such as accomplished by volitional control of emotion and activity; 2, a chemical reduction of bombardment with the use of sedatives; 3, a reduction of the formation of metabolic acids; 4, an alkaline diet; 5, a reduction of the availability of sugars; 6, cholinesterase; 7, drugs to lower the excitability of the axon hillock.

A differential reduction of cholinergic activity favoring the constrictor center and avoiding excessive impairment of cortical and other centers may prove to be a serious difficulty; nevertheless the great importance of hypertension and other neuropathological problems now call for a systematic application of the known principles of cholinergic physiology. Until these principles are more firmly established, empirical combinations of procedures 1 to 7 may also be in order.

SUMMARY AND CONCLUSIONS

1. The primary premise underlying the humoro-electrotonic theory of mediation is that acetylcholine is highly electrogenic at its site of liberation.

2. Synaptic potentials created by the liberation of acetylcholine in synaptic bombardment of a nerve cell body and its dendrites add their effects one to another and generate a common electrotonic current which flows from the dendrites to the axon hillock in the internal circuit and out through the axon hillock membrane back to the dendrites in the external circuit.

3. The membrane of the axon hillock is detonated at the site of emergence of the electrotonic current at a rhythm proportional to the intensity of the current.

4. The intensity of the current varies with the area of synaptic activity and the sum total of free electrogenic acetylcholine.

5. By virtue of the anticholinesterase activity of acid, physiological changes of cH create parallel changes in the sum total of free acetylcholine driving the nerve cells. Synaptic bombardment remaining constant, the acetylcholine pools attain higher or lower levels of concentration with increasing and decreasing cH, thus generating stronger or weaker electrotonic currents of proportional

motivating power. The greater summation and after-discharge produced by faradic stimulation of Hering's nerve during hypercapnia and the lesser effects during hypocapnia uphold this view. Observations collected from the older literature and from the recent experiments of Eccles add strong support.

6. The conclusion therefore seems warranted that carbon dioxide does not stimulate the respiratory center, but indirectly determines the effectiveness of prevailing synaptic bombardment.

7. It is proposed that acid-humoral mediation is the basic mechanism about which the details of the control of breathing were built.

8. As judged by evolutionary evidence acid-humoral mediation is a primitive mechanism of long standing whose development served to meet the needs of a continuing supply of energy.

9. The occurrence of acid-humoral mediation throughout the central nervous system and peripheral cholinergic systems in higher forms evidences a general retention of a mechanism originally designed for the primary purpose of providing energy.

10. Survival of the acid-humoral mechanism is thought to impress certain basic characteristics of nervous integration throughout the central nervous system.

11. Of these characteristics, electrotonic mediation is regarded as the most significant.

12. Summation of stimuli, after-discharge, intimately connected half-centers, dual excitatory afferents, reciprocal inhibition and precedence of stimulation would seem to be a natural outgrowth of humoro-electrotonic mediation.

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Since our paper has gone to press, V. M. Garasenko's interesting experiments on "The Use of Carbon Dioxide at Lowered Barometric Pressures" reviewed in Am. Rev. of Soviet Medicine, **2**:(2) 119–125, 1944 have come to our attention.

During flights at altitudes exceeding 10,000 meters above sea level, the partial pressure of O_2 and CO_2 in the alveolar air of fliers is decreased despite the use of oxygen equipment. Experiments have shown that the addition of 5 to 5.5% CO_2 assures vital activity even when the partial pressure of oxygen in alveolar air was only 30 mm Hg.

It was also found that the feeling of "well-being" and capacity to perform work at high altitudes was increased when the CO_2 was added to the O_2. The percentage of CO_2 should be progressively increased with the decrease in barometric pressure reaching 15–16% at 120 mm Hg.

The experiments of Garasenko support our contention that a controlled content of carbon dioxide in the tissues is as important to nervous function as is a controlled supply of oxygen.

STUDIES ON THE CARDIO-VASCULAR SYSTEM OF DOGS WITH RADIOACTIVE INERT GASES[1]

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The purpose of this investigation has been twofold. First, we have attempted to develop a technique whereby the exchange of radio-krypton and other inert gases may be accurately studied in dogs. Many experimental procedures are possible with animals which would not be possible with humans. Second, we have undertaken to study the effect of various factors on the uptake of gas.

METHODS. The dogs were anesthetised by nembutal, given intravenously to approximately the same degree of anesthesia. A tube was inserted in the trachea and rendered air-tight by inflating a rubber cuff. It was then connected to the metabolor containing the gas-oxygen mixture. The rate of gas uptake was then measured by means of a Geiger counter placed in contact with the right hind foot, both enclosed in a lead-walled chamber. The concentration of the gas-oxygen mixture was maintained at a constant level. The counting method was that which is standard for the use of radioactive gases.[2] Each run was continued for twenty minutes or longer (1, 2, 3).

Normal index values. As a criterion of gas uptake by the animal we used the "index" developed in the laboratory during the past three years. It consists of drawing a curve through the points obtained by plotting counts per minute against time, and taking the ratio of the number of counts at seven minutes to the number of counts at fourteen minutes. The reproducibility is as close as can be reasonably expected in mammalian experimentation. In tables 1, 2 and 3 are shown five normal indices obtained with each of seven dogs. The mean standard deviation equals 1.17 and the mean standard error equals 0.51. (The "probable error" equals 0.67×0.51 or 0.34.) One is, therefore, justified in ascribing significance to index differences in excess of three times the standard error, or greater than 1.5 index units. These data have also been treated by means of Fisher's Analysis of Variance. It was demonstrable that the mean dog values were definitely too highly significant with reference to individual variation of measurement. (The functions lay between the 1 per cent and the 0.1 per cent probability levels.) However, in order to err, if at all, on the side of conservatism, we have doubled this value and have not regarded any induced change in index value as valid unless it differs from the normal mean by at least three

[1] The work described in this paper was done under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the University of California.

[2] The technique involved in the biological application of radioactive rare gases has hitherto been confidential. Papers are, however, being submitted for publication by this laboratory at the present time. Several are now in press (1, 2, 3).

points. Within these limits the so-called 7/14 index appears to be a wholly valid and reliable guide to both differences between individual animals and to variations in the same animals when the experimental conditions are varied.

Variation of the normal index by experimental procedure. Since the rate of gas uptake in its early stages is generally considered to be determined largely by the cardio-vascular condition of the animal, our efforts have been directed mainly toward altering this factor. Various physical effects were first observed with results as shown in table 2. When the animal is infused with large quantities of normal saline there is a temporary increase in the volume of intercellular fluid. This increases the total amount of fluid which must be saturated with the

TABLE 1

	I	II	III	IV	V	VI	VII
	71.1	65.8	66.6	68.8	66.4	63.7	63.7
	68.7	67.2	64.3	69.5	66.3	63.6	62.5
	71.9	64.4	63.1	70.3	66.6	64.0	64.3
	69.3	66.6	64.5	68.0	68.6	63.7	61.0
	68.3	63.4	62.4	70.0	65.7	63.9	61.4
Mean	69.8	65.4	64.2	69.3	66.7	63.7	62.5
s.d.	±1.40	±1.39	±2.96	±0.69	±0.97	±0.03	±1.63
se.e.	±0.60	±0.59	±0.92	±0.30	±0.43	±0.01	±0.73

TABLE 2

TREATMENT	NORMAL INDEX	EXPERIMENTAL INDEX	PER CENT CHANGES
Hydration			
1 liter saline, intra v. after 1 hr.	64.3	57.2	−11.0
1 liter saline, intra v. after 1 hr.	69.6	62.2	−11.0
1 liter saline, intra v. after 1 hr.	69.9	62.6	−10.4
Temperature			
Whole body heated	69.5	72.7	+4.60
Foot in counter heated	64.3	72.0	+12.0
Body chilled (shivering)	69.5	63.2	−9.10

gas and therefore equilibrium is achieved more slowly. Consequently, the index will tend to be lower. With respect to temperature it is clear that warmth increases the vascular bed by dilatation of the small vessels and due to the increased blood flow affords a more rapid absorption of the gas. Conversely, the vasoconstriction induced by cold cuts down the blood flow, particularly in the extremity (foot) and restricts gas uptake.

Inasmuch as the rate of gas uptake is determined largely by the amount of blood flow to the extremity it seems reasonable that various drugs having definite cardio-vascular effects might thereby increase the rate of gas exchange and hence raise the index. Therefore drugs were given as shown in table 3.

Adrenalin. The administration of adrenalin is attended by significant lowering of the index. The cutaneous vaso-constriction with associated shivering of the animal permits less blood to be delivered to the extremity and, therefore, retards the gas uptake. These local effects apparently outweigh the other "favorable" cardio-vascular actions of the drug such as increased cardiac output,

TABLE 3

DRUG	NORMAL INDEX	EXPERIMENTAL INDEX	PER CENT CHANGE
Adrenalin, 2 cc. subcut	69.9	50.0	−28.5
Adrenalin, 0.5 cc. intraven	69.3	54.1	−21.9
$NaNO_2$, 01. gram intraven	56.8	54.8	−3.5
Histamine	64.3	57.9	−10.0
Dexedrine	69.5	64.7	−6.9
Caffeine, 50 mgm. per kilo, i.v	69.5	72.6	+11.6
Caffeine, 50 mgm. per kilo, i.v	64.3	73.9	+15.0
Caffeine, 50 mgm. per kilo, i.v., by mouth	63.1	73.3	+16.2
Theophyllin, 100 mgm. per kilo, i.v.	64.3	82.3	+38.1
Theophyllin, 100 mgm. per kilo, i.v	63.1	75.4	+19.5
Theophyllin, 100 mgm. per kilo, i.v..	69.5	76.3	+8.3
Theophyllin, 25 mgm. per kilo,			
measured after 2 minutes.	66.3	69.6	+4.9
measured after 15 minutes	63.6	67.0	+5.0
Theophyllin, 25 mgm. per kilo,			
measured after 1 hour	63.1	66.3	+5.0
measured after 3 hours	63.6	67.0	+5.0
measured after 24 hours	63.6	63.7	±0.0
measured after 48 hours	63.6	63.6	±0.0
Theophylline, 50 mgm. per kilo,			
measured after 15 minutes	66.3	74.2	+11.9
measured after 24 hours	66.3	66.4	±0.0
measured after 48 hours	66.3	66.3	±0.0
Theophylline, 10 mgm. per kilo,			
measured after 15 minutes	63.1	66.3	+5.0
measured after 24 hours	63.1	63.7	+0.8
measured after 48 hours	63.1	62.5	−1.0

elevated blood pressure, etc., the overall effect being one unfavorable to rapid gas exchange.

Sodium nitrite. When sodium nitrite is given in the dose of 0.1 gram intravenously the blood pressure drops and peripheral vasodilatation is marked, involving chiefly the postarteriolar beds. The resultant effect is one of relative peripheral circulatory failure which impairs the blood flow to the extremity and consequently retards the rate of gas exchange. Hence, despite an increase in the peripheral vascular bed the overall effect is unfavorable.

Histamine. The cardiovascular effect of histamine is similar to the nitrites. Despite peripheral vasodilatation, the blood pressure drops, cardiac output is not increased, and the result is one of impaired circulatory efficiency and hence unfavorable for rapid gas exchange.

Dexedrine. This drug, like adrenalin, is sympathomimetic in effect.

Xanthines. The xanthines exert certain cardio-vascular actions which result in increased peripheral blood flow: viz., the cardiac output is increased, blood pressure is maintained, peripheral vessels are dilated. It seemed, therefore, that these drugs would be likely to elevate the index. As seen in the accompanying table, theophylline and caffeine in varying doses and time relationships were administered by different routes. The results show a consistent and significant rise in the index with both of these drugs. In general, intravenous administration is followed by a progressive rise in the index as one increases the dose from 10 to 100 mgm./kilo. The duration of the effect lasts at least 3 hours. For detailed analyses of these data see the accompanying table.

SUMMARY

1. In anesthetised dogs the normal 7/14 index for the uptake of radiokrypton is both significant and reproducible.

2. Physical procedures and certain drugs which impair the peripheral blood flow consistently lower the index.

3. The xanthines, which tend to facilitate peripheral blood flow, uniformly raise the index. Dosage and time relations have been studied.

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SPREAD OF ACh INDUCED ELECTRICAL DISCHARGES OF THE CEREBRAL CORTEX

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Sjöstrand (1937) revealed that the application of acetylcholine (ACh) to the cortex previously treated with strychnine and eserine produced fast regular waves. Miller, Stavraky and Woonton (1940) reported that 1 per cent ACh solutions applied to the cortex produced no alteration but when the cortex had been previously eserinized spikes occurred similar to those induced by the application of strychnine to the cortex. Chatfield and Dempsey (1942) found 1 per cent ACh effective only when prostigmine had been previously applied to the cortex. Brenner and Merritt (1942) reported that higher concentrations of ACh (2.5 to 10 per cent) would evoke spike potentials from the cortex without the aid of eserine or prostigmine. Chatfield and Dempsey and Brenner and Merritt noted that the spike discharges induced by ACh tended to remain localized to the area of application. Brenner and Merritt noted on several occasions a spread of the discharge to a homologous area of the contralateral hemisphere. Chatfield and Dempsey indicated a spread of the fast, ACh induced, activity to an ipsilateral cortical area which was subsequently found to be fired by the application of strychnine to the original area of ACh application. These observations on the spread of the activity induced by the topical application of ACh to the cortex, suggested that when a spontaneous spread occurs it does so along neuronal pathways. The following studies were carried out to determine the validity of this premise and also to ascertain if strychnine applications would facilitate the spread of these ACh induced discharges.

METHODS. Thirty-two adult cats were employed for these studies. Anesthesia was induced by the administration of Dial with urethane[1] in doses of 0.45 cc. per kgm. Both cerebral hemispheres were widely exposed. Unipolar silver ball electrodes were employed as roving electrodes and bipolar electrodes were placed in stationary positions. Sensory areas of the cortex were selected and these were identified by their strychnine firing characteristics as outlined by Garol (1942). In addition there were occasional samplings of the temporal lobe, neuronal pathways being also identified by the strychnine technique. In the early experiments electrodes were scattered over both hemispheres, ACh applied to one portion of an area and the location of the ACh firing determined. When the ACh discharges remained localized strychnine was applied to the same area to which ACh had been applied and after adequate strychnine firing occurred ACh was reapplied. By this technique the factors in local firing, spontaneous spread along neuronal paths, and the facilitation of spread by strychnine were determined.

[1] We wish to thank Ciba Pharmaceutical Products for the Dial with urethane.

In order to determine the nature of the secondary ACh discharge by attempting to propagate the discharge to a third area the following technique was followed: A battery of four pairs of electrodes was applied to each of the probable primary and tertiary areas of ACh firing and roving grounded electrodes were used on the proposed secondary ACh firing area. Electrodes were placed on adjacent and distant areas. Strychnine was placed on the secondary area and firing of the tertiary area determined. As the primary area was usually homologous and contralateral in most instances, the region of the primary area fired by the secondary would also cross fire. Firing from the primary to the secondary area was then checked. The optimum timing of the two applications of strychnine to the secondary and primary areas and of ACh to the primary area was determined. Care was taken in each experiment also to sample areas not in neuronal connections so that any tendency to spread along the cortical feltwork might be apprehended.

In all experiments 3 per cent strychnine nitrate solution was used. This was applied by means of small squares of filter paper, 1 to 2 mm. square. As soon as a given strychninization produced adequate spikes, the pledget was removed. ACh was used in solutions varying from 5 to 25 per cent. These solutions were applied on pledgets varying from 2 to 3 mm. square and were removed, unless some special indication existed, as soon as adequate ACh firing occurred. Recording was by means of a 3 channel Grass electroencephalograph.

Results. The application of ACh to the cortex in the manner described resulted in a diminution of the electrical activity of the cortex. Within a period of time ranging from 5 to 20 minutes there appeared an increase of the electrical activity of the cortex. This increased activity consisted of rapid, intermittent discharges of increased voltage. (ACh discharges.)

For the most part the ACh discharges remained sharply localized to the region of the application of the drug, usually failing to fire even adjacent or more distant portions of the same cortical area. A spontaneous spread was encountered in two instances, both times to a contralateral homologous area and in one of these instances an ipsilateral area was also fired (fig. 1). Subsequent strychninization proved that the areas to which the ACh discharge had spread were neuronally and dromically fired by the primary area.

Attempts to facilitate the propagation of ACh discharges by application of strychnine to an area undergoing ACh discharge succeeded only in disrupting the firing in the primary area. The reversal of the procedure, i.e., strychninization followed by application of ACh, resulted in the propagation of ACh discharges to the areas fired by strychninization (fig. 2). These secondary ACh discharges, whether spontaneous or induced by strychninization, differed in certain respects from the primary ACh discharges. Frequently the secondary discharges did not occur with the earliest primary discharges nor did they continue until the final discharges from the primary area. The secondary discharges were usually of lower voltage and between the discharges a considerable amount of normal cortical activity was apparent.

Since most of the primary applications of ACh were made to areas 5 and 7,

the projection pathways to the opposite hemisphere from the areas under investigation were by way of the corpus callosum. When the secondary area of ACh firing was contralateral, section of the corpus callosum abolished the secondary firing leaving the primary discharge intact (fig. 3).

In attempting to propagate the ACh discharge from a secondary to a tertiary area, the application of strychnine to an area undergoing secondary ACh firing was found quickly to break up the secondary ACh discharge leaving only strychnine spikes. Again a reversal of applications was attempted, with strychninization

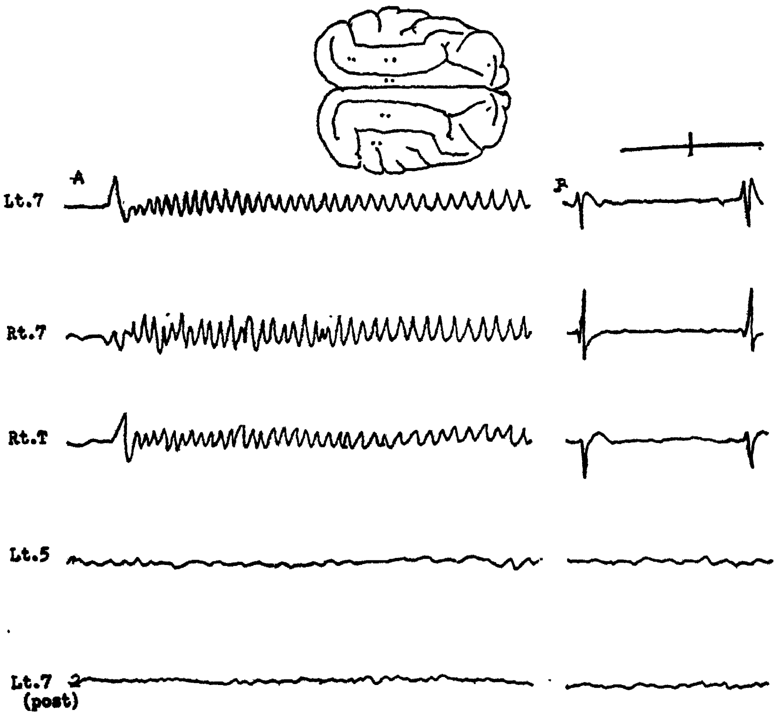

Fig. 1. Spontaneous spread of ACh discharge dromically and along neuronal pathways. A: ACh discharge occurring in Rt. 7, Rt. T. and Lt. 7 following application of ACh pledget to Rt. 7. B: Strychninization of Rt. 7 fires the same areas as did ACh. Horizontal Marker, 1 sec. Vertical Marker 500 μ volts. Same in all figures.

tion of the secondary area firing the tertiary area, followed by strychninization of the primary area and finally application of ACh to the primary area. While in all, 22 attempts were made to propagate the ACh discharge from the primary to a secondary and thence to a tertiary area, in only two instances was this accomplished. In these two instances the tertiary ACh discharge bore the same relationship in onset, duration, voltage and intervening cortical activity to the secondary discharges that these bore to the primary (fig. 4).

Conclusions. The local application of ACh to the exposed cerebral cortices of cats under Dial with urethane anesthesia resulted in a diminution of electrical activity. The nature of the diminution of the electrical activity is not yet clear.

Following the diminution of electrical activity rapid, high voltage intermittent electrical discharges occurred. These have been designated ACh discharges.

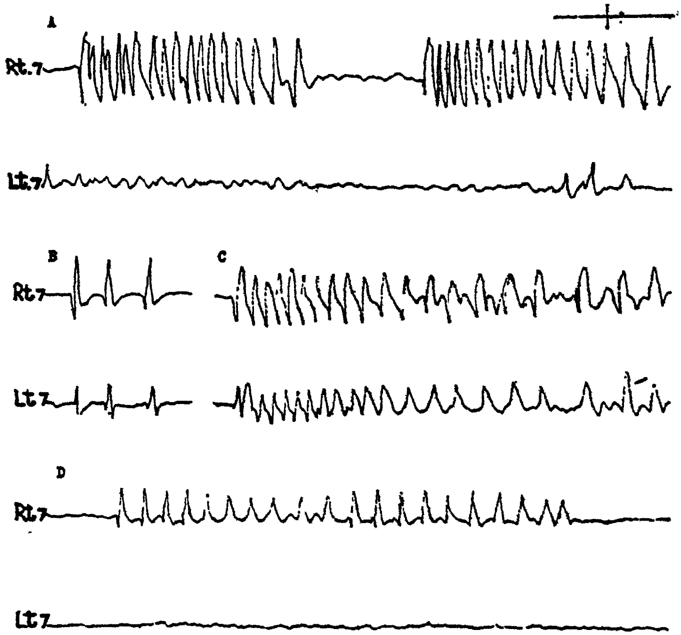

Fig. 2. Strychnine facilitation of the spread of ACh discharge. A: localized ACh discharge resulting from application of ACh to Rt. 7. B: strychninization of Rt. 7, firing opposite 7. C: ACh applied to Rt. 7 during strychninization resulting in spread of ACh discharge to opposite 7. D: Repetition of A 2½ hours later showing again localized discharge.

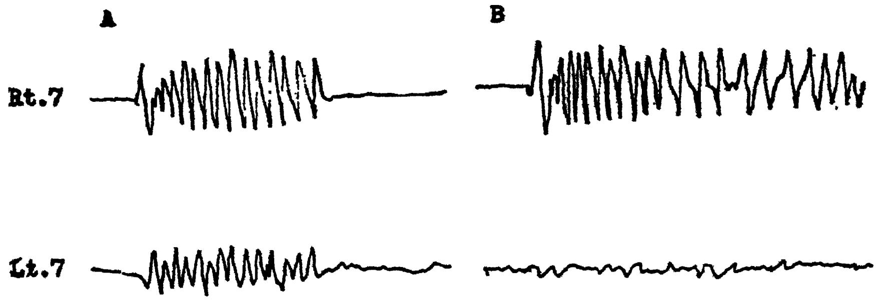

Fig. 3. Effect of section of corpus callosum on contralateral secondary ACh discharge. A: primary ACh discharge in Rt. 7 with secondary ACh discharge in Lt. 7. Spread induced by strychninization. B: Disappearance of secondary ACh discharge following section of the corpus callosum.

The present studies confirm those of previous authors in the observation that ACh discharges tend to remain sharply localized to the region of application. The observation of Brenner and Merritt that ACh discharges occasionally spread spontaneously to a small area of the opposite hemisphere suggested the spread is

along an anatomical structure. This is proven by the section of the corpus callosum with obliteration of the secondary discharge when the primary discharge arises from an area which projects through the corpus callosum. Since ACh discharges which spread spontaneously do so dromically and only to areas fired by strychninization of the primary area the anatomical pathway must be

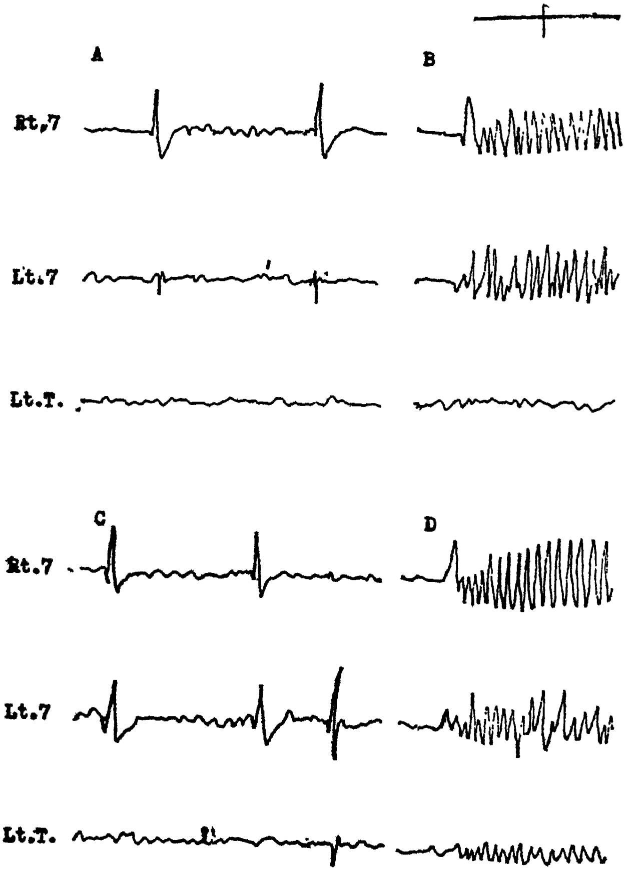

Fig. 4. Spread of ACh discharge from primary to secondary, and from secondary to tertiary area. A: Strychninization of Rt. 7 fires contralateral 7 but not contralateral area of temporal lobe. B: ACh and strychnine firing of Rt. 7 produces secondary ACh discharge in Lt. 7. No ACh discharge in temporal. C: Strychninization of Rt. 7 and Lt. 7. Rt. 7 fires Lt. 7 and Lt. 7 fires Lt. T. D: ACh application to Rt. 7 after C with ACh discharges as follows: primary in Rt. 7, secondary in Lt. 7 and tertiary in Lt. T.

neuronal. The two instances in which it was possible to propagate the discharge to a tertiary area suggest that the discharge in the secondary area is not an axonal discharge obtained from the axones of the primary neurones, but rather is a discharge of the neurones of the secondary area. The infrequency with which tertiary firing was accomplished may well have been due to the difficulty in timing of the three applications, necessitated by the deleterious effects of primary strychnine spikes on either primary or secondary ACh discharges.

Strychninization prior to ACh application resulted in a facilitation of the spread of the ACh discharge. While the mechanism for this is not self evident, it is conceivable that strychnine firing may function in a manner similar to that of a single shock stimulus in controlling cortical clonic responses (Rosenblueth, Bond and Cannon, 1942). There was also some indication that a prior application of strychnine enhanced the primary ACh discharge. In many instances there was greater regularity, faster frequencies and higher voltages in the primary ACh discharges when a previous strychninization occurred and compared with a simple application of ACh to the same area prior to strychninization.

Differences between primary and secondary, and secondary and tertiary ACh discharges were chiefly the diminishing amplitude of the discharge and the degree of intervening cortical activity between discharges. These variations are consistent with the neuronal concept of the spread of the discharge. Thus one region or one area of the cortex does not project en masse to another area nor does one area receive projection fibers only from another area. Thus while the application of a drug to the primary area will conceivably affect all the available neurones in that region, only a portion of these will project to the neurones of a given secondary area and of these only a still smaller proportion will project to the tertiary area. Hence the driven ACh discharge may well be expected to decrease and normal cortical activity increase in proportion to the number of steps in the propagation.

SUMMARY

The application of ACh to the cortex resulted in a diminution in the electrical activity which was rapid in onset. This was followed by the appearance of ACh discharges which varied in type, and tended to remain sharply localized to a small region. Spontaneous spread of ACh discharges occurred along anatomical structures and followed in dromic fashion, neuronal paths. Secondary ACh discharges were possibly neuronal and not axonal discharges. Certain differences between primary, secondary and tertiary ACh discharges were discussed.

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A PROGRESSIVE PARALYSIS IN DOGS CURED WITH SYNTHETIC BIOTIN

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In 1927 Boas (1) observed pronounced spasticity of the limbs in rats suffering from the effect of feeding egg white, now known to induce a biotin deficiency (2). In 1942 Nielsen and Elvehjem cured this neurologic condition with crystalline biotin (3).

Between 1919 and 1921 Voegtlin and Lake (4), Karr (5) and Cowgill (6) observed, among other symptoms, a progressive paralysis in dogs on diets probably lacking in most of the B complex factors. Treatment with natural sources of the B vitamins, such as yeast, rice polish, wheat germ, tomato juice—all good sources of biotin as well as the other factors, resulted in prompt reversal of the paralytic process and loss of the other symptoms.

In the present study (7) a progressive paralysis has been produced quite consistently in dogs on a vitamin B complex free diet supplemented with 8 synthetic factors of the B complex, including vitamins B_1 and B_6. Under these conditions the paralysis was not complicated by such symptoms as vomiting, acute anorexia, related to a vitamin B_1 deficiency (8, 9, 10), and convulsive seizures, related to a deficiency of vitamin B_6 (11–16), which were observed by the earlier investigators (4, 5, 6). Consequently, it was possible to study in more detail the development of the paralysis and its reversal on treatment with synthetic biotin.

EXPERIMENTAL. Forty-six dogs (43 puppies and 3 adults) were used. All were housed in separate cages and given access to food and water at all times. Only healthy animals known to be growing at a normal rate were placed on experiment after being freed from intestinal parasites and immunized against distemper.

Puppies from 6 to 10 weeks old were kept on an adequate yeast-containing diet from weaning until their hemoglobin levels had reached values of 13 to 14 grams/100 cc. Previous work in this laboratory (17) has shown that puppies treated in such a manner survive longer on deficient diets, and thus the early deaths so disastrous in experiments of this kind are avoided. As a result of this procedure, the animals were started on the experiment at different ages but probably at a more constant nutritional level.

Diets. Chart 1. I. *Adequate diet.* The diet fed the positive controls contained brewers' yeast at a level of 10 per cent, replacing an equivalent amount of sucrose in the basic diet (BD). On this regime the dogs remained normal in every way and experienced vibrant health and vitality throughout the experimental period, which ranged from 1 to 1½ years.

CHART 1

Diets

CONSTITUENTS	BASIC DIET (BD)	YEAST DIET (YD)
	per cent	*per cent*
Casein (acid and alc. extd.)	40	40
Sucrose	36	26
Cotton seed oil	18	18
Cod liver oil	2	2
Salt mixture[1]	4	4
Brewers' yeast[2]	0	10
Total	100	100

Vitamin supplements given to both experimental animals and controls weekly:

Vitamin K—5 mgm. 2 methyl 1-4 naphthoquinone

Vitamin E—¾ cc. Mixed tocopherols (Lederle)

Vitamin A, D } 100 drops of cod liver oil conc. yielding { 200,000 U.S.P. u. vitamin A; 27,000 u. vitamin D

8 synthetic factors of B complex

1. Salt mixture: bone meal, steamed, 57.8%, sodium chloride 24.4%, lime stone (oyster shell flour) 12.2%, iron sulfate 3.7%, magnesium oxide 1.2%, copper sulfate 0.3%, manganese sulfate 0.1%, zinc oxide 0.1%, cobalt carbonate 0.1%, potassium iodide 0.1%.

2.

	Vitamin content of brewers' yeast (sample 2019) *micrograms/gram*	and *yeast extract (type 300)* *micrograms/gram*
Thiamin	700	600
Riboflavin	60–70	200
Pyridoxine	40	100–120
Nicotinic acid	540	1500
Pantothenic acid	170	500
Biotin	2.4–2.7	0.7
Inositol	3800	3000

II. *Deficient diets.* All animals on deficient diets were fed the basic, vitamin B complex free diet (BD) supplemented with 7 or 8 of the synthetic vitamin B complex factors (SV); namely, thiamin, 1.4 mgm.; riboflavin, 0.7 mgm.; nicotinic acid, 6 mgm.; pantothenic acid, 6 mgm.; vitamin B_6, 6 mgm.; para-amino-benzoic acid, 6 mgm.; inositol, 100 mgm.; and choline, 100 mgm. per dog per day. These were dissolved in distilled water and administered orally to each dog once or twice a week. The fat soluble vitamins A, D, E and K were also fed orally to

each dog in addition to the vitamins A and D contained in the cod liver oil of the basic diet (chart 1).

The experimental grouping, dietary regimes and incidence of paralysis are shown in chart 2.

1. *Diet lacking only in the unknown or commercially unavailable factors of the B complex (XV): BD + SV − XV.* The dietary regime is expressed as BD (basic diet) + SV (8 synthetic B vitamins) − XV (additional vitamin factors of the B complex unknown or commercially unavailable) to emphasize the fact that we are dealing with a multiple B complex deficiency of unknown quantity and quality. We do know quite accurately the number of B complex factors present but not the number or physiological properties of all the missing ones.

CHART 2

Incidence of paralysis

DIETARY REGIME	NO. OF DOGS	NO. BECOMING PARALYZED
1. BD + SV − XV....	16	15
2. BD + SV − (PAB + XV)	2	0
3. BD + SV − (PA + XV)...	4	2
4. BD + SV − (NA + XV)........	2	1
5. BD + SV − (Cho. + XV) ..	2	1
6. BD + SV − (B_6 + XV).......	9	2
7. BD + Y or YD Positive controls.	11	0
Total....	46	21
Dogs on BD + 7 B factors	19	6 (32%)
Dogs on BD + 8 or more B factors ...	16	15 (94%)
Dogs on YD (Positive controls)...	11	0 (0%)

BD = basic (B complex free) diet.
SV = 8 synthetic vitamin factors of the B complex.
XV = the rest of the B complex vitamins either unknown or commercially unavailable.
YD = yeast diet consumed by positive controls.

The most clear-cut syndrome and much the highest incidence of paralysis occurred on this regime.

Most of the puppies continued to grow for a short period, depending on their growth impulse at the time they were placed on the deficient diet. The weight then remained constant for most of the remaining experimental period.

The paralysis. One of the first signs observed in what may be termed the prodromal period is an apprehensiveness or state of tension. The animals over-react to every stimulus. A generalized weakness develops which often expresses itself in the quadruped by a hanging of the head which is so marked that vision is obscured. Due to this, the dog wanders in an apparently aimless manner, running into objects and acting as though his mental faculties were impaired. The dog soon learns, however, to compensate for this by twisting the neck up and back with a rotary motion, thus bringing the head into a position of

minimum strain. This sign tends to come and go and has always disappeared before the onset of the final progressive paralysis.

During the prodromal period there may occur one or more bouts of transitory generalized paralysis, from which the animal recovers spontaneously without treatment. Such attacks are characterized by marked spasticity of the hind legs which causes great difficulty in walking, but rarely is there complete loss of function.

The length of the prodromal period shows considerable variation. In most of the animals it extended over a period of from 2 to 6 months although in 2 animals it was not observed at all. The onset of the progressive paralysis was very sudden in these 2 cases and occurred while the animal appeared in an excellent state of nutrition.

Male dogs usually developed priapism during the last part of the prodromal period, and in some animals incontinence of urine was observed.

The onset of the progressive paralysis is usually sudden and unpredictable as to the exact time of occurrence in spite of the warning prodromal symptoms. There is a wide variation in time of onset, varying from $7\frac{1}{2}$ to 48 weeks on the deficient diet. Suspicion is aroused when these abnormally alert dogs tend to sit most of the time. This so-called alertness may be a type of psychomotor restlessness; it has been observed in all the dogs during the prodromal period. The sequence of events in the progressive paralysis leads next to marked spasticity of the hind legs and then to inability to get up from a sitting position. At this stage it is hard to distinguish the progressive paralysis from the transitory bouts because the hind legs are involved in these also. The time element is important in differentiating the two; in the early attacks loss of function never extends over an appreciable time and never involves the forelegs. After the hind legs are paralyzed completely, the animal begins to have trouble with the forelegs. There may be a lag of 4 to 8 hours before this occurs. Awkwardness due to spasticity is observed first, then a complete inability to use the forelegs. The dog often struggles violently to get up at this stage but is forced by his condition to lie prone. The animal may remain apparently stationary at this stage for 1 to 4 hours; then the neck begins to fail and later there is complete loss of function in the neck. At this stage the paralysis has become much more flaccid, and the dog usually lies with legs sprawled to either side and with the head lying flat in the midline. If untreated, the paralysis progresses to the point where the respiratory mechanism is involved and the animal dies from respiratory failure.

If treatment is started in time, recovery is in the reverse order: function returns to the neck first, then the forelegs and lastly the hind legs.

The one animal, no. 5, of this group of 16 dogs (table 1) failing to show any paralysis became infested with intestinal parasites and died of very severe anemia after 29 weeks on the diet, having a red blood count of 1,540,000, hemoglobin of 3 grams per 100 cc. and a hematocrit of 10 volumes per cent.

Of the 15 dogs developing paralysis, 2 showed only transient attacks. One, no. 6, died of lung abscess after 2 transient attacks during the prodromal stage,

TABLE 1

Dogs on regime BD + SV − XV

DOG NO.	INITIAL AGE	INITIAL WT.	T. P.		F. P. P.		TREATMENT	RESPONSE
			No. of attacks	Time on diet	No. of attacks	Time on diet		
	wks.	*kilo.*		*wks.*		*wks.*		
1	10	6.6	4	38, 39 42, 46	0		B. Y.	C. R.
2	10	3.7	1	6	1	7.5	None	D. P.
3	41	9.8	0		1	27.0	None	D. P.
4	17	5.0	0		1	30.0	None	D. P.
5	17	6.3	0		0		None	D. A.
6	17	4.7	2	12, 19	0		None	D. L. A.
7	20	7.5	1	33	1	38.5	B. Y. E.	C. R.
8	20	8.2	1	45	1	48.0	None	D. P.
9	29	15.7	0		1	22.0	Biotin	R. P.
10	29	7.3	0		2	17.5 17.7	Biotin Biotin	R. P. R. P.
11	29	8.4	0		1	16.5	None	D. P.
12	29	13.4	1	18	3	16.5	Biotin Biotin Biotin B. Y.	R. P. R. P.* R. P. C. R.†
13	37	11.4	0		1	18.5		P. R. P. D. C. F.
14	34	9.1	4	22, 23 25, 31	1	35.0	Biotin B. Y.	R. P. C. R.
15	35	9.1	4	8, 10 24, 28	1	30	Biotin B. Y.	R. P. C. R.
16	31	11.1	0		1	15	Biotin	P. R. P. D. C. F.

Initial—Time at which dogs were placed on deficient diet.

T. P.—Transient paralysis
B. Y.—Brewers' yeast
C. R.—Complete recovery
D. P.—Died of paralysis
D. C. F.—Died of cardiac failure
D. A.—Died of anemia
F. P. P.—Final progressive paralysis
B. Y. E.—Brewers' yeast extract
R. P.—Recovery from paralysis only
P. R. P.—Partial recovery from paralysis
D. L. A.—Died of lung abscess

* Maintained on synthetic biotin for 10 days.
† Died of cardiac failure 16 weeks after yeast was discontinued.

and another, no. 1, was treated with yeast after 4 such attacks. This treatment resulted in complete recovery from all neurologic signs and fairly rapid return to normal health and vitality.

Of the 13 dogs remaining in this series, all developed the typical progressive paralysis. Five of these received no treatment, and all of these died within 12 to 18 hours of the onset after passing through the consecutive stages as outlined.

Attempts were made then to reverse the process with therapy. The first trial was with a water extract of yeast (chart 1). Dog 7 was selected for this test after the paralysis had progressed to the stage of complete loss of function in the hind legs and early involvement of the forelegs. It had been observed previously that the transient attacks from which the animals recovered spontaneously involved only the hind legs; hence, this involvement of the forelegs served to differentiate this attack from one in which spontaneous recovery could be expected. The dog was given 8.5 grams of a water extract of yeast in powder form about 5 p.m. In addition, the dog had the basic, B complex free, diet replaced by one containing this same yeast extract at a level of 10 per cent. The extract was given in gelatin capsules, the first few being forced down the animal's throat. As soon as the dog tasted the extract, some of which stuck to the outside of the capsule, she showed an abnormal desire for it and ate ravenously all the 14 capsules offered. She was then replaced in the cage at 5 p.m. and allowed to remain overnight. The next morning at 9 a.m. she was standing and able to walk in a fairly normal manner, but was very weak and looked a little dazed. At first there was some residual spasticity, but after 6 weeks on the diet all traces of abnormal neurologic signs had disappeared. The dog was normal, healthy and had unusual vitality. She was later placed on the control diet and has remained in excellent condition for 65 weeks (fig. 1).

All of the 7 remaining dogs were treated with biotin. At the time most of these dogs developed the progressive paralysis they had been depleted of several of the B complex factors in addition to biotin and were lacking in energy and general resistance. Of the 7 paralyzed dogs treated with biotin, only one, no. 12, seemed in really good general condition, having marked vitality and strength. This dog developed the progressive paralysis 3 times, and each time was cured of the paralysis by synthetic biotin alone in the following total doses: (1) 1000, (2) 500 and (3) 1000 micrograms. The biotin was made up in physiological saline in a concentration of 50 micrograms per cc., sterilized by autoclaving for 15 minutes and injected *subcutaneously* in the following partial doses: (1) 100 micrograms every 2 hours for 10 hours, plus a final dose of 500 micrograms; (2) 50 micrograms every 2 hours for 10 hours, plus a final dose of 250 micrograms, and (3) 200 micrograms every hour for 5 hours. In the first and third relapses the results of treatment were entirely satisfactory, but in the second the results were not quite so good. Marked residual weakness and neck symptoms, as seen in the prodromal period, remained after treatment was completed. An attempt then was made to maintain the dog on 50 micrograms of biotin per day, but this amount proved insufficient. When the amount was increased to 100 micro-

grams, it appeared to be adequate although at times there was slight arching of the neck which was interpreted as indicating a borderline maintenance dose. The plan was to continue the dog at this level for 10 days, then increase to 200 micrograms; but on the tenth day the dog relapsed for the third time with a very rapidly progressive paralysis, which again responded to treatment with 1 mgm. of synthetic biotin (fig. 2). Because of the severity of this attack and the fact that the dog came so very near dying in this and a previous attack, it was decided to place her on the yeast-containing control diet for a month in order to build her up. Sixteen weeks after the yeast was discontinued, while she was in the prodromal stage with occasional neck symptoms and spasticity of the extremities, she died

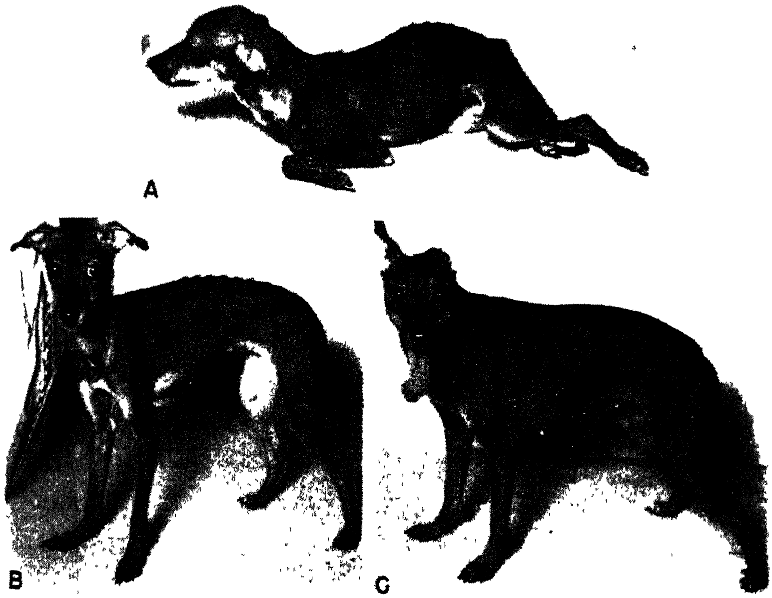

Fig. 1. Dog 7.
A. Paralyzed state. B. Seventeen hours after receiving treatment with a water extract of yeast. C. After 8 months on diet containing the yeast extract.

suddenly of cardiac failure. At 9:30 a.m. on the day of her death she jumped out of the cage as she frequently did at such times, ran around the room a few times, was caught and returned to the cage. She appeared normal in every way except for mild prodromal signs noted above. At 2 p.m., when next observed, she was dead but still warm. An autopsy was performed immediately. The tongue was extremely cyanotic. There was marked dilatation of the heart, the right side being more dilated than the left. In addition, there was pronounced congestion of the liver and intestines. All of this suggested cardiac failure as the cause of death. Histological sections of the organs were normal except for a mild interstitial nephritis in the kidneys and marked congestion in the liver and intestines.

The 6 other dogs treated with biotin were much weaker and less able to respond to any specific therapy. However, one of these animals, no. 10, extremely weak when treated, recovered completely from the paralysis but relapsed within 12 hours after biotin therapy was discontinued. He responded again to biotin treatment to the point where he could stand and walk, but was so weak after this second attack that it was decided to sacrifice him for tissue studies.

Another dog, no. 9, the third dog treated with biotin, showed a specific response in the cure of the paralysis but died 5 days later of a necrotic lesion of the shoulder.

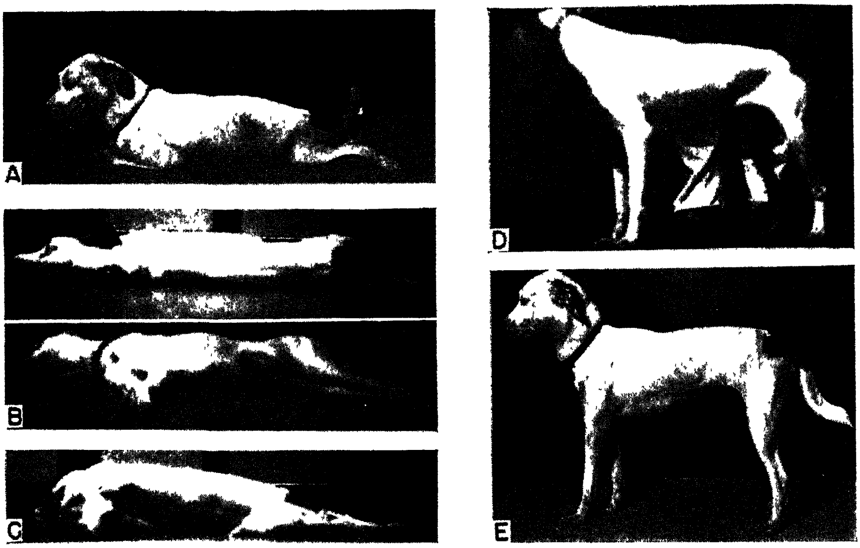

Fig. 2. Dog 12 in third relapse, showing stages of progressive paralysis and complete recovery with biotin treatment with the time relationships. Total dose, 1 mgm. (200 micrograms at 4, 5, 6, 7 and 9 p.m.).

8/18/44: A. 4 p.m.; B. 6 p.m.; C. 11 p.m. 8/19/44: D. 3 a.m.; E. 10 a.m.

The fourth dog, no. 16, treated with biotin was one in which we expected excellent results because she did not appear to be as much affected by the other deficiencies of the diet in addition to biotin. This dog reached the point of complete paralysis in the hind legs, then the forelegs and then the neck. These stages were very clear-cut and distinct. Approximately 8 hours after the biotin treatment was started, during which time the animal had continued to get worse, there was a definite reversal of the process. Function first returned to the neck; then the forelegs regained their function completely and the dog was able to sit up. There seemed to be very slight improvement in the hind legs. At this point the dog became very impatient and tried to propel herself about by means of the forelegs. This she succeeded in doing, but the exertion proved too much for her at this stage of recovery and she died suddenly in cardiac failure in less than 15 minutes. An electrocardiogram made about 4 hours before death showed no characteristic changes from the normal except for sinus arrhythmia which is quite common in dogs (18).

The fifth dog, no. 13, also died in a heart attack after function had returned to the neck only, following biotin therapy. This dog was treated with prostigmin (2 mgm. total dose) given subcutaneously over a period of 1 hour. It resulted in marked increase in salivation, lacrimation, urination and defecation, indicating physiologic reaction from the drug, but it had absolutely no effect on the paralysis. Biotin treatment was started approximately 1 hour after the prostigmin was discontinued.

The sixth dog, no. 15, showed a specific response to biotin in the cure of the paralysis. This dog was in an extremely weakened condition at the onset of the progressive paralysis. He suffered complete loss of function in the 4 extremities and in the neck. This was restored by the biotin treatment to the point where the animal could stand and walk, and the priapism which had existed also disappeared. He was still extremely weak and had no interest whatsoever in the basic diet; but when offered 10 grams of the brewers' yeast used in the control diet, he ate it ravenously, even in his exhausted state. He was offered another 10 grams which he consumed and was then placed on the yeast diet to restore some of the other missing factors before he was again depleted.

The seventh dog, no. 14, treated with 200 micrograms of biotin every hour for 5 hours, was in the same weakened condition as no. 15, and his progress on treatment followed practically the same pattern. This animal also showed a peculiar appetite for the yeast where none existed for the basic diet. The yeast was given only after the paralysis had responded specifically to the biotin treatment.

It seemed of interest at this point to determine whether the omission of any single entity from the group of 8 synthetic vitamins, SV, would accentuate or hasten the onset of the specific neurologic syndrome.

2. *Diet lacking para-amino-benzoic acid (PAB) in addition to XV: BD + SV − (PAB + XV).* Two adult black dogs were maintained on this regime for 28 and 27 weeks respectively without neurologic changes, depigmentation of the fur or any other sign of deficiency (19, 20). These 2 animals were then also deprived of pantothenic acid, another B complex factor associated with achromotrichia (21, 22), for an additional period of 30 and 26 weeks respectively. At the end of this time there was likewise no depigmentation of the coat in either dog, but both developed characteristic neurologic signs. One showed a transient attack in the prodromal period from which it recovered spontaneously and died suddenly of unknown cause about 12 weeks later. The other developed the progressive paralysis which presumably was the cause of death.

3. *Diet lacking pantothenic acid (PA) in addition to XV: BD + SV − (PA − XV).* Two puppies were placed on this regime for 11 and 17 weeks respectively, when they died suddenly of unknown cause without evidence of neurologic change or depigmentation of the coat.

4. *Diet lacking nicotinic acid (NA) in addition to XV: BD + SV − (NA + XV).* Of the 2 puppies placed on this regime, one died of unknown cause after 6 weeks on the diet, showing slight loss of weight but without signs of black tongue or other deficiency symptoms. The other developed black tongue after 14 weeks on the diet and was cured with synthetic nicotinic acid (1 mgm./kilo. per

day for 10 days). Six weeks later the dog developed black tongue again and was cured a second time, but in this case with a larger dose (10 mgm./kilo. per day for 10 days). One week later, while the treatment was still going on, the dog began to show prodromal signs and experienced a transitory attack, from which he recovered spontaneously. Two weeks later, before the dog would normally have exhausted his store of nicotinic acid (23), he died of the progressive paralysis.

5. *Diet lacking choline (Cho.) in addition to XV: BD + SV − (Cho. + XV).* Of the 2 dogs on this regime, one died quite suddenly of unknown cause after 29 weeks on the diet. Since at this time the dogs were not examined with much regularity for signs of paralysis, it is quite possible that it could have died either of the rapidly progressive paralysis or of congestive heart failure. The other dog developed the typical paralysis without obvious complications and identical in every way with the symptoms shown by the dogs receiving 8 or more synthetic factors. Rats ingesting a diet containing casein at a level of 30 per cent or more do not require choline (24, 25). If this is true of dogs, this regime is, for all intents and purposes, identical with the one BD + SV − XV.

6. *Diet lacking vitamin B_6 in addition to XV: BD + SV − (B_6 + XV).* Of the 9 dogs (8 puppies, 1 adult) on this regime, 7 died presumably of the microcytic hypochromic anemia characteristic of vitamin B_6 deficiency (11, 15, 17). The other 2 dogs both became paralyzed after 17 and 23 weeks respectively, one before there was objective evidence of vitamin B_6 deficiency and the other after the dog began to show blood changes characteristic of the hypochromic microcytic anemia with a red blood count of 6,850,000, hemoglobin 9.5 grams per 100 cc. of blood and hematocrit 32.7 volumes per cent. In the latter case the paralysis was complicated by the presence of convulsive seizures which have been described as occurring in animals on a B_6 deficient diet (11–16). In the former case the neurologic disorder occurred before vitamin B_6 deficiency was evident and, consequently, was unaccompanied by convulsive seizures. Even so, the syndrome was slightly atypical with respect to the rapidly progressive paralysis seen in the dogs on the regime BD + SV − XV in that it failed to progress through all the consecutive stages. Vitamin B_1 was given subcutaneously (10 mgm.) to rule out the possibility of a failure to absorb the material given orally. This had no discernible effect. Since the animal was getting rapidly worse, treatment with vitamin B_6 was started and continued for 3 weeks. During this time the paralysis appeared to progress less rapidly than before, but there was definitely no reversal of the process. The general condition improved slightly at first, then gradually got worse. It must be remembered that the dog remained paralyzed and at practically the same stage during all this period. Of all the 19 animals becoming paralyzed, in not a single instance except this one did the paralysis become arrested at any given stage. Because the general condition was getting worse and the paralysis no better, the basic diet was replaced by the yeast diet. On this regime the general condition improved amazingly; function returned to the forelegs but the spastic paralysis of the hind legs remained unchanged. There was marked exaggeration of the reflexes, clonus was present to an inexhaustible degree and different groups of muscle fibers showed fibrillation and later atrophy.

There were coarse tremors. The histological sections, examined by Dr. Valy Menkin, showed vacuolization and degeneration of some of the ventral horn cells and suggestive evidence of damage in the region of the lateral cortical spinal tract. Further studies on this latter point, however, are in progress.

Biotin assays. Microbiological determinations of biotin have been made on dietary constituents, the tissues of depleted and normal dogs and their urinary and fecal excretion. The method employed is that of Snell and Wright (26) for nicotinic acid, altered as required for biotin assay. The complete data are being prepared for publication at a later date. Ten assays on the urine of 5 dogs on the regime BD + SV − XV average 2.96 micrograms per 24-hour excretion period as compared with 14.62 micrograms, the corresponding value for the positive controls on the yeast diet (27).

Discussion. In the present study a characteristic neurologic syndrome occurred consistently on a diet lacking several factors of the B complex. It is not prevented by 10 of the known synthetic B complex factors; namely, vitamin B_1, riboflavin, nicotinic acid, vitamin B_6, pantothenic acid, para-amino-benzoic acid, inositol, choline, folic acid and xanthopterin. It is prevented and cured by brewers' yeast and a water extract of this brewers' yeast, both known to contain biotin. It is also cured in a few hours with synthetic biotin in a total dose as small as 500 micrograms. The fact that synthetic biotin in such very small amounts will give a specific response so quickly makes it reasonable to believe that biotin is concerned in the etiology.

That the highest incidence of the neurologic disorder occurs in animals receiving the largest number of B complex factors is interesting but not surprising. We know that the addition of riboflavin to rat diets brought out the vitamin B_6 deficiency dermatitis in much higher instance and more characteristic form than diets lacking riboflavin (28). The same thing was true of the chick dermatitis due to pantothenic acid deficiency (29). Recently Darby has produced rickets in rats in a more consistent and characteristic form by adding choline to the Steenbock rickets-producing diet (30). The addition of a vitamin factor not concerned in the etiology of a syndrome often brings out the syndrome in more clear-cut form. Hence, it is reasonable to believe that none of the 8 synthetic factors used regularly in the dietary regimes described above are concerned in the etiology of the resulting neurologic picture.

The nature of the defect in this neurologic syndrome is not yet understood. The complete failure of any response to prostigmin given parenterally in adequate amounts leads us to believe that the syndrome is not due to a defect in the acetyl choline metabolism. A degenerative lesion of any significance in the central nervous system is quite unlikely because of the rapidity of recovery.

The possibilities of a toxic effect, disturbed potassium metabolism or disturbed creatin metabolism remain. The first possibility has not been investigated at all. With respect to the potassium metabolism, one dog treated with potassium chloride solution administered subcutaneously in 100 mgm. doses every hour for 7 hours showed little or no response whereas a definite response was obtained later with biotin.

Others have observed paralysis on B complex deficient diets in which synthetic

factors were employed. In 1936 and later in 1938 Morgulis *et al.* (31) described a muscular dystrophy in rabbits which was due to a lack of 2 factors—one fat soluble, later identified as vitamin E, and the other water soluble, presumably a member of the B complex group. More recently, in 1945, the B complex factor has been identified by Milhorat and Bartels (32) as inositol. Their observations suggest that tocopherol forms a condensation product with inositol in the gastro-intestinal tract (tocopherol-inositol ether) and that the inherent defect in muscular dystrophy is a deficiency in this reaction of condensation.

Morgan, in 1941, described a "progressive flaccid paralysis" in dogs on different types of vitamin B complex deficiency (33). The paralysis developed in 3 of her 4 deficient dietary regimes. Since 2 of these were more or less opposite in character, it is reasonable to believe that a common factor lacking in both diets was responsible for the neuromuscular syndrome.

In 1942 Wintrobe *et al.* (34) observed the development of abnormal gait in pigs on a diet lacking either pantothenic acid or vitamin B_6. This was accompanied by sensory neuron degeneration. Protection against these changes was afforded by the inclusion of these 2 factors in the diet.

Hogan (35) has observed in chicks a paralysis which in many ways seems similar to the syndrome in dogs just described. In the chick, however, the anti-anemia factor, B_c, is the protective or curative agent. The paralysis in the chick is described as a "spastic cervical paralysis." "At the onset the neck is extended and rigid, as in a turkey that has been alarmed and is searching to see if danger is near. The attacks are at first intermittent, but later they become continuous. The wings are slightly drooped and quiver. The poult chirps continuously as if in pain. . . . When the poult is moribund, the body seems to relax and frequently the neck is twisted." Nine poults developed the cervical paralysis on a diet containing crystalline biotin. All were given a source of vitamin B_c. The time required for recovery varies according to the method of administration; but in 2 poults given 100 micrograms of crystalline vitamin B_c intraperitoneally, one recovered in 4 hours and the other in 6. Vitamin B_c was not supplied as such to any of the dogs developing the paralysis in the present study, but its absence did not interfere with a specific response of the neurologic syndrome to biotin.

The cardiac damage resulting in the sudden death of 3 of the dogs appears to be different from that occurring in vitamin B_1 deficiency since there was no variation from the normal either in the electrocardiogram or in histologic sections of the heart. In the latter deficiency, certain well-established pathological changes occur, namely, focal and diffuse myocardial necrosis and pronounced electrocardiographic changes (36).

It has been shown previously (37, 38) and confirmed in the present work that 8 synthetic factors, SV, are completely inadequate as a source of the B complex for puppies. Brewers' yeast, however, introduced at a level of 10 per cent in the basic diet proved entirely adequate as a source of the B vitamins. This diet, consumed by the controls, resulted in vibrant health throughout the experimental period which in the case of 2 controls and 1 yeast-treated dog lasted for well over a year. Diarrhea was not observed in a single instance in spite of the high yeast content of the diet.

SUMMARY AND CONCLUSIONS

Evidence is presented that a characteristic neurologic syndrome of progressive paralysis occurs consistently in dogs on a vitamin B complex deficient diet supplemented with thiamin, riboflavin, niacin, pyridoxine, calcium pantothenate, inositol, para-amino-benzoic acid and choline in adequate amounts.

The signs of this neurologic syndrome are alleviated by biotin in relatively small therapeutic doses, 100 micrograms per kilo of body weight, in a comparatively short time. Consequently, we conclude that biotin is concerned in the etiology of this syndrome in dogs.

The nature of the physiologic defect is not clear at the moment. The speed of cure makes it unlikely that a degenerative lesion in the nervous system occurs, and failure to respond to prostigmin would seem to rule out a defect in the acetyl choline metabolism.

Cardiac damage resulting in sudden death was observed in 3 dogs. These heart lesions differed from those described as a result of vitamin B_1 deficiency in that the latter showed pronounced electrocardiographic changes and pathological alterations while these showed neither.

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THE STIMULATING EFFECT OF ACETYLCHOLINE ON THE MAMMALIAN HEART AND THE LIBERATION OF AN EPINEPHRINE-LIKE SUBSTANCE BY THE ISOLATED HEART

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In a series of experiments on the heart-lung preparation (dog) we observed that large doses of acetylcholine (ACh) produce, in the atropinized preparation, an improvement of the heart action associated with a marked increase of the coronary sinus flow. The effects are very similar to those observed when epinephrine is given. The stimulating effect of ACh is abolished by nicotine as well as by ergotamine. These facts seem to show that ACh has, in addition to its well known "muscarine-like" depressor effect, a stimulating action which can be described as "nicotinic." We therefore assume that this neurotransmitter acts on nervous structures, located in the heart, which are equivalent to sympathetic ganglia or chromaffine tissue.

As far as we know, this effect of ACh has not been described, but there are some experimental indications that the mammalian heart possesses sympathetic ganglionic structures. This hypothesis has been formulated by Dixon (1924) who observed that nicotine has positive ino- and chronotropic effects on the isolated heart. It seemed of interest to study in greater detail the response of the heart to ACh, since the action of this neurotransmitter substance on sympathetic intracardiac structures might possibly reveal an important peripherally localized mechanism of cardioregulation.

In later experiments, in order to obtain completely isolated hearts, we chose the Langendorff preparation, which has the advantages of an extremely simple technique, and because the organ survives in good condition in spite of being perfused with physiological salt solution (Tyrode). This last fact enabled us to detect by simple means the liberation of an adrenalin-like substance in the perfusate of the heart.

METHODS. For the heart-lung preparation, dogs from six to eleven kilograms were used. The anesthetic was morphine-chloralose. Each preparation was perfused with a total of 1.5 to 2 liters of defibrinated blood from a second dog, mixed with the blood of the heart donor. Aortic pressure was recorded with a mercury manometer, venous pressure with a water manometer, and aortic and coronary flow by a method previously described (13) which permits continuous recording of the flow on smoked paper.

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ACh and epinephrine were injected close to the heart into the cannula inserted into the superior vena cava, and the other drugs into the venous reservoir.

For the Langendorff preparation more than 100 hearts of cats, rabbits and guinea pigs were used. They were perfused with Tyrode solution warmed to 38° and saturated with O_2 (95 per cent) and CO_2 (5 per cent). Glucose (0.1 per cent) was added. The solution was introduced under pressure into the coronary system by means of a cannula inserted into the aorta a few millimeters above the aortic valves. At the beginning of the experiment the pressure was adjusted to obtain an optimal amplitude of ventricular contractions. The amount of salt solution flowing through the coronaries was recorded with the flow meter mentioned above. An isometric lever (1:10) attached to the heart apex recorded the amplitude of the heart beats.

To test for the presence of an epinephrine-like substance in the perfusate of the Langendorff heart, the coronary outflow was collected in samples of the same volume, before, during and after the action of ACh or nicotine, and tested for its activity on the following biological preparations: the hypodynamic frog's heart (*rana pipiens*), the isolated rectal cecum of the fowl, and the isolated small intestine of the rabbit.

The frog hearts were prepared according to Straub, using a modified cannula recommended by Krayer and co-workers (15) which permits continuous renewal of the solution acting on the heart without alteration of the hydrostatic pressure. The hearts were prepared at least 5 hours before starting the actual experiment and were irrigated with Tyrode solution made isotonic by adding 3 parts of distilled water to 7 parts of the salt solution.

Samples of the perfusate of the mammalian heart were collected from time to time, made isotonic for the frog, cooled to room temperature, and then used successively for perfusion of the Straub heart. The samples generally had a slight stimulating action. The addition of ACh or of nicotine "in vitro" to the atropinized perfusion fluid has never shown an epinephrine-like effect on the frog heart, either in the present series or in previous experiments conducted in Chile on the hearts of the toad (Bufo Chilensis Gay) and the bullfrog (Calyptocephalus Galli).

The jejunum or ileum of the rabbit and the rectal cecum of the chicken were suspended in a moist chamber immersed in a water bath at 38° and were irrigated drop by drop with the perfusate collected from the mammalian heart. This technique is very similar to that recommended by Gaddum and co-workers (11), the only difference being that the O_2-CO_2 mixture was directed into the moist chamber from below. In all cases the fluid irrigating the intestine was changed without altering either the frequency of the drops or the temperature.

The usual reaction of the atropinized intestine of the rabbit to high concentrations of ACh is a slight increase of the amplitude of the pendular movements, but is never a decrease in tone. However, it is not unusual for the atropinized rectal cecum of the fowl to relax under the influence of ACh. This would seem to indicate that some of these preparations contain a certain amount of nervous and perhaps chromaffine tissue which can release epinephrine when stimulated

by ACh or nicotine. These preparations can be utilized for the test if nicotine is added to the irrigating fluid. This drug abolishes, as might be expected, the "nicotinic" effect of ACh on the rectal cecum, but does not change (in concentrations up to 2 mgm. per cent) the reaction of the organ to epinephrine. The following drugs were used: epinephrine hydrochloride, atropine sulfate, basic nicotine or nicotine tartrate (expressed as basic nicotine), ergotamine tartrate, curare and acetylcholine chloride. The curare was an extract of a crude preparation from Venezuela (5 mgm./liter Tyrode). As to the acetylcholine chloride, it is important that the solution is not acidified, because the stimulating effect of ACh does not appear in the presence of acid.

RESULTS. 1. *The stimulating effect of acetylcholine on the mammalian heart. The heart-lung preparation.* No details of the experiments on the heart-lung preparation need be presented, as the subsequent experiments on the Langendorff preparation are more clear-cut and completely confirmatory, but the results of the thirty-two heart-lung experiments can be summarized as follows:

When small doses of ACh (0.1–0.2 mgm.) are given, they show a typical "muscarinic" action consisting of a brief standstill of the heart, which is manifested by a sudden fall of the arterial and rise of the venous pressure, accompanied by a decrease of the aortic flow. The coronary flow shows a steep but very brief rise. After atropinization (10–50 mgm.) small doses of ACh produce no effect, but larger doses (5–10 mgm.) produce an intense epinephrine-like action which is shown by an increase of the heart rate and aortic flow, a decrease of venous pressure, and a long-lasting increase of coronary flow. After ergotamine (4–8 mgm.) has been added to the blood, ACh in the same doses loses completely its epinephrine-like effect on the heart. Nicotine in doses of 1 to 10 mgm. produces a very marked effect on the dynamic conditions of the atropinized heart: increase of rate, decrease of venous pressure, and increase of aortic and coronary flow. In most of the experiments, a second injection of the same dose of nicotine had no detectable effect, and following a large dose of nicotine the injection of large doses of ACh also regularly produced no stimulating effects.

Therefore, we conclude that large doses of ACh show, after atropinization, epinephrine-like actions on the myocardium and the coronaries. This stimulating effect is abolished, on one hand, by the action of nicotine, on the other, by ergotaminization of the heart.

Experiments on the Langendorff preparation. Fundamentally the same results were obtained from the cat, guinea pig and rabbit hearts perfused with physiological salt solutions. Before giving atropine the classic depressor effect of small doses of ACh was observed. If the dose of ACh was increased, the negative chrono- and inotropic effects became more marked, but generally this phase of depression was followed by a period of increased frequency and amplitude. After giving atropine (1 mgm./liter) ACh, given in relatively large doses (0.05–0.2 mgm.), produced, with surprising regularity, its epinephrine-like effect, i.e., increase of frequency and amplitude.

In the Langendorff preparation the coronary vessels do not react to ACh in a uniform way. There are marked differences from species to species as well as

from animal to animal. The same is true for the action of epinephrine. It is possible to show, however, that the following rules are valid: 1, small doses of ACh show a "muscarinic" action, which can be abolished by atropine; 2, large doses of ACh cause a change in the coronary flow which is similar to that observed when small doses (0.1–1 microgram) of epinephrine are injected into the aortic cannula; 3, the epinephrine-like effect of ACh is especially pronounced after atropine, which prevents the appearance of the initial muscarine-like action which generally occurs; 4, in a given preparation the "nicotinic" action of ACh on the coronaries is always identical with that of epinephrine (fig. 1).

The following experiments represent typical examples of the action of ACh:

The abolition of the stimulating effect of ACh by nicotine. Figure 1 shows a record of an experiment performed on a perfused cat's heart illustrating the ventricular contractions and changes in coronary flow. In A, a typical depressor effect of ACh (10 micrograms) is observed, which, in this case, is accompanied by

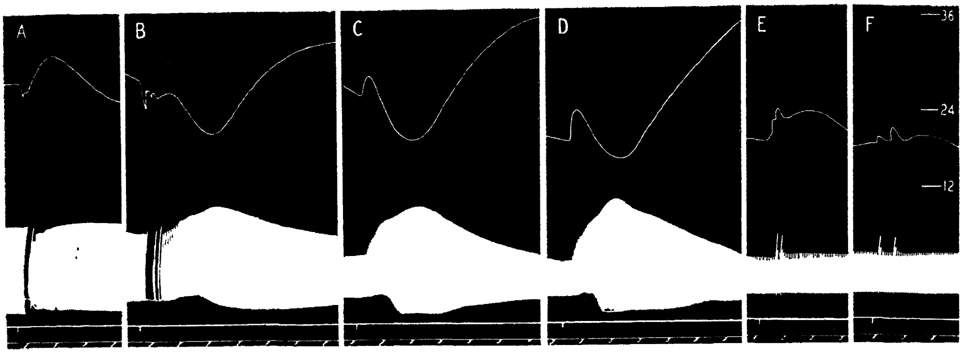

Fig. 1. Isolated cat's heart. Lower tracing: Ventricular contractions. Upper tracing: Continuous record of coronary flow, calibrated in cc./min. Time: 15 sec. For description see text.

an increase in coronary flow. In B, a large dose of ACh (50 micrograms) is injected with the result that the inhibitory effect is more pronounced, but is followed by an increase of amplitude. The coronary flow is now diminished, ("nicotinic" effect). Between B and C, the preparation is atropinized (1 mgm./liter), and in C, the dose of 50 micrograms of ACh is repeated. Now that the depressor action of ACh is absent, the heart reacts with a more pronounced positive ino- and chronotropic action, which can be called "nicotine-like" in view of the fact that it is extremely similar to that of nicotine (0.2 mgm.), injected in D into the aorta. The nicotinization was maintained by adding nicotine (2 mgm./liter) to the perfusion fluid of the heart. The effect of ACh, injected a few minutes later in E, and of nicotine given in F, is now completely abolished.

Figure 2 represents an experiment performed upon the heart of a guinea pig which illustrates *the abolition of the stimulating effect of ACh by ergotamine.* While 50 micrograms of ACh, injected in 1 show only a marked depressor effect and a nearly maximal dilatation of the coronaries, a larger dose (100 micrograms) given in 2 produces an initial "muscarinic" effect followed by an increase of amplitude.

The reaction of the coronaries to this relatively large dose of ACh is manifested by an initial dilatation; subsequently a slight contraction appears, which is observed in the dropping of the flow beneath the initial level. In order to show the reaction of the heart to epinephrine, 0.1 and 0.5 microgram are given in 3 and 4 respectively, and an increase of amplitude and rate, as well as coronary constriction, are observed. Between 4 and 5 atropine is added to the Tyrode solution perfusing the heart and in 5 the same dose of ACh as given in 2 is repeated. The reaction is very similar to that obtained with epinephrine in 4. Between 5 and 6 ergotamine is added to the perfusing fluid (10 mgm./liter), and here the injection of ACh in 6 has almost no detectable effect. But the preparation still responds to the injection of epinephrine (0.1 microgram) in 7, and only after very long-lasting (30 min.) ergotaminization, which markedly damages the heart, can it be seen in 8 that the effect of epinephrine (0.1 microgram) on the coronaries is completely abolished, although the myocardium is still slightly affected. In all our experiments it was impossible to completely eliminate with ergotamine the effect of epinephrine. Even when the heart was severely damaged by large

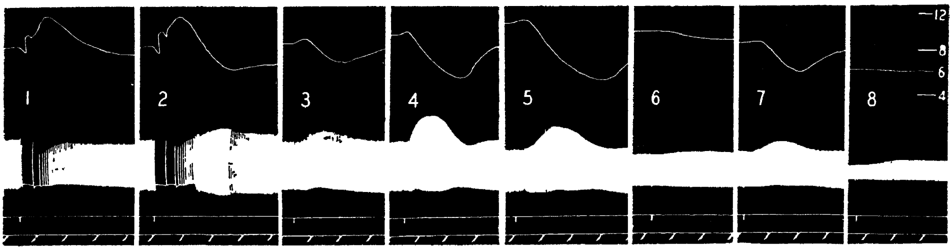

Fig. 2. Isolated guinea-pig's heart. Lower tracing: Ventricular contractions. Upper tracing: Continuous record of coronary flow, calibrated in cc./min. Time: 15 sec. For description see text.

doses of ergotamine, it still responded to epinephrine, while the "nicotinic" action of ACh was easily eliminated with relatively slight ergotaminization.

In another series of experiments (not illustrated) it was shown that the "nicotinic" action of ACh on the atropinized heart is readily abolished by adding curare (5 mgm./liter) to the perfusion solution of the heart.

2. *Demonstration of the release of an epinephrine-like substance in the heart under the stimulus of acetylcholine and nicotine.* The hearts of cats and rabbits were perfused with Tyrode solution, as described above, and their coronary outflow was tested for its epinephrine-like activity. Figure 3 shows an example of an experiment performed on an atropinized cat's heart (upper record). To detect the epinephrine-like activity of its perfusate, a hypodynamic Straub heart was used (lower record). In 1, the frog's heart is perfused with diluted and cooled perfusion fluid from the cat's heart (P.F.C.H.) collected during A. A slight increase of the amplitude is observed. In 2, 0.1 mgm. of ACh is injected into the cat's aorta; the perfusion fluid is collected during B, diluted, cooled and given in 3 to the Straub heart, which reacts with a marked positive inotropic action. At the height of the action the heart is washed, *x*, with "normal"

P.F.C.H., that is, fluid collected during a period in which no drug was injected into the cat's heart. In 4, a sample of P.F.C.H., collected during C, is given to the frog's heart. In 5, the frog's heart is perfused with "normal" P.F.C.H. to which 2 mgm. of nicotine have been added "in vitro" to 20 cc. The direct action of nicotine on the frog's heart causes a fairly pronounced depression. After washing, x, with "normal" P.F.C.H., the frog's heart recovers. In 6, nicotine (2 mgm.) is injected into the cat's aorta, the perfusion fluid is collected during E, diluted, cooled, and given in 7 to the Straub heart which reacts with a

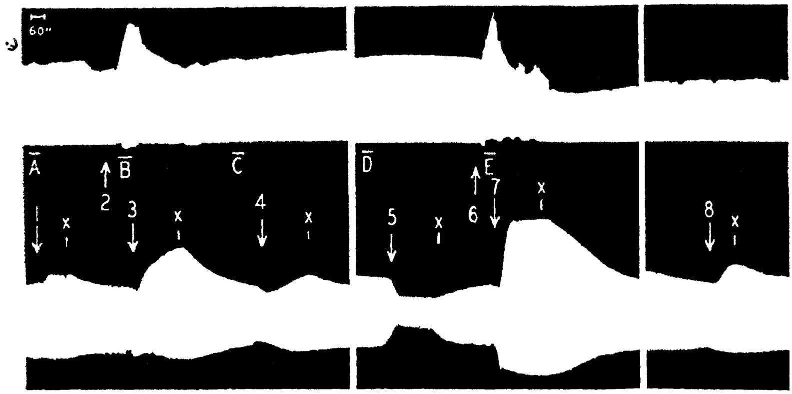

Fig. 3. Upper record: Ventricular contractions of an isolated cat's heart. Lower record: Ventricular contractions of hypodynamic frog's heart. For description see text.

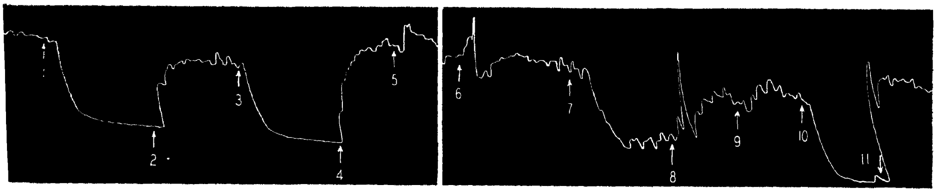

Fig. 4. Isolated rectal cecum of a chicken, irrigated with Tyrode solution collected from the outflow of a perfused cat's heart. For description see text.

very pronounced positive inotropic action. The activity of the perfusate is considerably greater than that corresponding to an epinephrine concentration of 2×10^{-9}, which is given in 8.

Figure 4 is a record obtained from an experiment on the isolated rectal cecum of the fowl, irrigated with the perfusate of an isolated cat's heart. First the organ is irrigated with "normal" P.F.C.H. (atropine 0.5 mgm. per cent). In 1, 20 cc. of the P.F.C.H., collected during the action of 0.5 mgm. of ACh, is given to the test organ, which reacts with a pronounced relaxation. In 2, the preparation is again irrigated with "normal" P.F.C.H., to which ACh has been added (0.5 mgm. per 20 cc.) in order to maintain approximately the same concentration of ACh as before. The organ recovers its normal tone. In 3, the same solution that was used in 2, but containing, in addition, epinephrine in a concentration of

10^{-8}, is given. The cecum shows a typical reaction to this drug which is quantitatively very similar to that produced by the heart perfusate collected during the action of ACh. In 4, the preparation is washed with "normal" P.F.C.H. containing ACh, and in 5 with P.F.C.H. which, this time, does not contain ACh, and it can be seen that this change does not significantly modify the intestinal tone. The perfusate from the cat's heart under the action of nicotine (2 mgm.) is collected in two samples of 20 cc. each. The first sample is given to the cecum in 7 and the second in 10, and both show a pronounced epinephrine-like effect; 8 and 11 show the effect of washing with "normal" atropinized P.F.C.H.

Previously it has been mentioned that after atropinization, some cecum preparations occasionally respond to ACh or nicotine with a relaxation. To show that this phenomenon does not interfere in this case, nicotine (0.2 mgm. per 20 cc.) was added "in vitro" to an atropinized "normal" P.F.C.H. and given to the preparation in 6. Only a relatively slight contraction, followed by a brief relaxation, occurred. (Often the first reaction of the cecum to nicotine is much more marked than in this case.) In 9, the intestine was irrigated with the same fluid as used in 6, and this time no perceptible nicotine effect could be seen.

Discussion. The positive ino- and chronotropic effects of ACh on the atropinized heart of various species of animals have been found without exception in more than 100 experiments. The action of this drug on the contractions of the heart and the coronary response are extremely similar to that of epinephrine. This observation suggests that ACh acts on certain intracardiac structures and stimulates them to liberate epinephrine.

We are able to exclude the possibility of a direct stimulating action of ACh on the heart muscle by indirect and direct evidence. The indirect evidence is based on the experiments in which the action of nicotine, curare and ergotamine were studied. As has already been observed by Dixon, nicotine has a strong stimulating effect on the heart beat, and our experiments, in addition, show an epinephrine-like effect on the heart vessels. If the injection of nicotine is repeated, it is observed that the stimulating effect becomes much less or is completely abolished. When the nicotinization is maintained by adding nicotine to the solution which perfuses the heart, the injection of ACh also loses its stimulating effect.

Curare, which is also known to block synaptic transmission, likewise abolishes the stimulating action of ACh. Finally, ergotamine, a substance which is known to desensitize certain effectors to the action of epinephrine or to sympathetic stimulation, prevents the heart from responding to the stimulating effect of ACh. This last effect, especially, supports the belief that, under the influence of ACh and nicotine, an epinephrine-like substance is released in the heart.

The direct evidence that, under certain circumstances, there actually is a liberation of epinephrine by the heart, consists of the following facts: the perfusate from the mammalian heart collected during the action of acetylcholine or nicotine produces a positive inotropic effect on the hypodynamic frog's heart, a relaxation of the rectal cecum of the fowl, and a decrease in tone of the small intestine of the rabbit.

The fact that under certain conditions an easily detectable amount of epinephrine is released in the mammalian heart suggests the possibility that the nervous structures responsible for this release might play a rôle in the normal mechanism of the regulation of the heart function. With the advance of our knowledge concerning humoral transmission, the opinion becomes more firmly established that, wherever acetylcholine plays a rôle as a transmitter of nervous impulses, epinephrine acts to modify its effects. We know, for instance, that epinephrine improves the synaptic transmission (4) and in high concentrations inhibits it (17, 4).

The presence of an adreno-sympathetic apparatus in the heart might possibly explain several problems regarding the nervous regulation of heart activity. One might assume, for instance, that the phenomenon of "vagal escape" is due to the release of epinephrine, which could be caused by ACh acting on the sympathetic structures in the heart. As Feldberg and Krayer (9) have shown, ACh is liberated and appears in considerable amounts in the coronary blood during vagal stimulation. Dale and his collaborators (7) attempted to explain the readiness with which the cat's heart escapes from vagal inhibition as due to the presence of cardio-accelerator fibers in the vagus nerve of these animals. It has been found repeatedly, in various species of animals, that stimulation of the vagus after atropine (12) and after sympathectomy (1, 2, 14) leads to an acceleration of the heart. This observation is generally accepted as evidence of the presence of accelerator fibers in the vagus. Numerous and elaborate suggestions have been made as to the location of the cell bodies of such fibers. It seems to us that the presence within the heart of nervous structures capable of releasing epinephrine would provide a more plausible explanation, i.e., the intracardiac sympathetic structures, sensitized by denervation, are stimulated by the ACh liberated during vagal stimulation. In any event, it seems necessary to investigate anew the problem of mixed motor and inhibitor vagal mechanisms.

The interpretation which we give to our experimental results does not agree with the findings of Cannon and Lissák (6) who showed that extracts of sympathetically denervated cat hearts did not exhibit the clear epinephrine effect (which was observed with extracts of normal hearts) on the blood pressure, the hypodynamic frog heart, the denervated iris, and nictitating membrane. These authors attribute their observation to the exclusive presence of post-ganglionic epinephrine-containing fibers in the heart, and they maintain, in the discussion of the paper, that there is no evidence for the presence of chromaffine tissue in the heart. However, they state that the extracts of the sympathetically denervated hearts, while having no effect on the blood pressure of the spinal cat or on the hypodynamic frog heart, still exhibit a slight epinephrine-like effect on the denervated iris and nictitating membrane; but these effects are regarded as being due to some other substance, rather than to epinephrine.

Perhaps the acceleration of the chronically denervated heart, which has been used as an indicator of epinephrine secretion from the adrenal gland due to fright, cold and other stimuli, should be interpreted as due in part to the epinephrine liberated by sympathetic structures in the heart itself.

So far, it has not been possible for us to obtain from the more recent literature morphological evidence in support of the hypothesis of an adreno-sympathetic apparatus in the heart. The ganglion cells which have been described in the heart muscle tissue usually have been considered as belonging to the vagal system. With morphological methods, however, it is not possible to decide whether or not some of these ganglion cells are connected with sympathetic fibers (16).

The presence of chromaffine tissue in the heart could provide a morphologic basis for the phenomenon of epinephrine liberation under the action of ACh. It is a well known fact that the chromaffine cells of the adrenal medulla are innervated by preganglionic fibers and belong embryologically as well as functionally to the same tissue as the ganglion cells of the sympathetic lateral chain. Under the stimulus of ACh the chromaffine medulla cells release epinephrine (10). Other chromaffine cells of the body may behave in the same way to similar stimuli. Thus it is believed that the chromaffine cells present in sympathetic ganglia are responsible for the liberation of epinephrine which is produced by stimulation of the ganglion with ACh (3).

There are a few older papers in which the presence of chromaffine tissue in the heart has been described. Trinci (18) showed the existence of such formations in the hearts of guinea pigs, cats and other mammals, and Wiesel (19) described the "Paraganglion cardiacum," supposed to consist of chromaffine tissue and located near the origin of the left coronary artery of the human heart. Busacchi (5) made a more detailed investigation of the distribution of chromaffine tissue in the human heart and described it as being located in the neighborhood of both coronary arteries, and especially well developed near the left.

We are unable to decide whether, in the case of the heart, we deal with ganglionic sympathetic structures, with chromaffine tissue, or with a combination of both. The evidence which we have obtained, however, supports the assumption that such structures in the heart do exist and play a rôle in the regulation of its activity.

SUMMARY

The injection of ACh into the isolated atropinized hearts of dogs, cats, rabbits and guinea pigs regularly causes an epinephrine-like effect, i.e., an increase of heart rate and amplitude, as well as a change of blood flow in the coronary vessels.

The positive ino- and chronotropic effects of ACh are abolished by ergotamine, by curare, and by nicotine.

The pharmacological characteristics of the stimulating action of ACh on the heart enable us to designate it as "nicotine-like."

In the perfusate of the isolated heart, it is possible to identify the presence of an epinephrine-like substance liberated when ACh or nicotine is injected into the heart. Under the stimulus of these drugs the perfusate acquires an activity which can be demonstrated by its positive inotropic action on the hypodynamic frog heart and by a relaxing effect on the rectal cecum of the fowl and on the small intestine of the rabbit.

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THE EFFECT OF HEMORRHAGE AND REPLACEMENT ON THE APPARENT VOLUME OF PLASMA AND CELLS

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There is satisfactory evidence that extravascular fluid is added to the circulation within a short time following blood withdrawal (Adolph, Gerbasi and Lepore, 1933; Price, Hanlon, Longmire and Metcalf, 1941). In studies on controlled hemorrhage which are reported elsewhere (Lawson and Rehm, 1945a, b), we obtained data on the apparent rate of fluid entrance at various stages of a continuous exsanguination. Plasma volume was measured as the dilution volume of the dye T-1824 at the beginning of the bleeding. The amount of dye removed in the bleeding was measured, and certain assumptions were made regarding the rate at which the remaining dye disappears into the tissues during the exsanguination. Estimates were obtained in this way of the total circulating dye at intervals during the bleeding, and were used, along with plasma dye concentration, for estimating the circulating plasma volume. The volume of fluid added to the circulation during the bleeding was calculated by subtracting the initial plasma volume from the sum of the volume drawn plus the volume remaining at the end.

Circulating cell volumes were estimated from the plasma volumes and the percentage of cells in drawn blood. The reliability of such estimates is open to question, since the percentage of cells in certain parts of the cardiovascular system may be quite different from that in drawn blood. The experiments of Fåhreus (1929) suggest that blood occupying the smaller vessels may have a much lower hematocrit than the blood flowing through them. If any large part of the measured plasma volume occupies vessels of this sort, our methods should overestimate the circulating cell volume. Evidence for such an error has been obtained by Stead and Ebert from bleeding and reinjection experiments on splenectomized dogs (1941). Our studies employed intact animals, and were done in three stages: 1, an almost complete exsanguination; 2, injection of whole blood or cell-free fluids; 3, complete exsanguination. Circulating cell volumes were estimated at the beginning and end of each stage and at intervals during each. The data suggest that the error is masked by contraction of cell reservoirs during the first stage, but not thereafter.

METHODS. All observations were made on barbitalized intact dogs, three hours or more after the induction of anesthesia. Blood was drawn from a femoral artery by free bleeding in unit volumes of 2 cc./kgm. at intervals of two minutes. The first bleeding was discontinued when mean arterial pressure, recorded from the other femoral artery, remained for two minutes below 20 mm. Hg. Heparinized autogenous blood or gelatin solutions were then injected,

usually in a volume of 36 cc./kgm. The second bleeding was begun approximately four hours later, and was continued at the same rate as the first until mean arterial pressure remained permanently below 10 mm. Hg.

Either 10 or 20 mgm. of the dye T-1824, depending upon the size of the dog, were injected twenty minutes before starting the first bleeding, and a second injection, usually 10 mgm., was given twenty minutes before starting the final bleeding. In selecting the dose to be given an attempt was made to keep the plasma dye concentration below 0.04 mgm./cc. so as to avoid rapid disappearance (Rawson, 1943). Blood samples were taken at the beginning, the end, and at intervals during the bleedings for plasma dye concentration and hematocrit determinations, care being taken to flush the cannula and collecting tube before sampling. These samples were drawn as part of the bleeding procedures, without interfering with the routine.

Plasma dye concentration was determined with the Klett-Summerson photoelectric colorimeter. Hematocrits were measured by centrifuging for one hour at 2600 r.p.m. in a head with a radius of 20.0 cm. measured to the bottom of the centrifuge tubes. The hematocrits were corrected for trapped plasma by subtracting 8 per cent of the relative cell volume as suggested by the data of Chapin and Ross (1942). The mean corrected hematocrits were used for computing the drawn volumes of cells and plasma. The amount of dye withdrawn was calculated from the volume of drawn plasma and its mean dye concentration.

On the first bleeding approximately one hour elapsed between the dye injection and the withdrawal of the last sample. On the final bleeding the interval was usually somewhat less. For these fairly short intervals it seemed safe to assume that our animals, if undisturbed, would lose dye at the rate of approximately 10 per cent of the injected dye per hour, as found by Gregersen and Rawson (1943). The total circulating dye at the start of the first bleeding (one-third hour after the injection) was accordingly taken to be 0.967 $\times$ the amount injected. At the start of the final bleeding the total circulating dye was calculated as $0.967 \times (D_1 + D_2)$, where D_2 is the new dye injected, and D_1 the dye remaining in the circulation from the first injection. Methods for estimating D_1 are given below. Plasma volumes were calculated from the general formula D/C, where D is the estimated total circulating dye at the time of sampling, and C is the plasma dye concentration in mgm./cc. Plasma volume at the start of the final bleeding was calculated from the special formula $\frac{0.967\ D_2}{C_2 - C_1}$, where D_2 is the amount of dye injected twenty minutes earlier, C_1 the plasma dye concentration immediately before the injection, and C_2 the concentration at the start of the bleeding. Residual circulating dye just before the new dye injection was calculated as $D_1 = C_1 \times \frac{0.967\ D_2}{C_2 - C_1}$.

It is not possible to predict, *a priori*, how the type of hemorrhage employed will affect the rate of dye disappearance into the tissues. Assuming that the rate remains at 10 per cent per hour of the total, as in undisturbed animals, we estimated the circulating dye at any time during the bleeding from the formula

$D_0 - d - 0.00167\ t\ \frac{(2\ D_0 - d)}{2}$, where D_0 is the dye present at the start of the bleeding, d is the dye withdrawn in the bleeding, and t is the duration of the bleeding in minutes at the time of the determination. Since dye is withdrawn in these experiments at a fairly rapid rate, the amount of dye which could disappear diminishes rapidly. Approximately the same values for dye disappearance were obtained when more elaborate and rigorous calculations were substituted for the crude formulation above.

Results and discussion. *Plasma volume.* Data are summarized in the form of average values in figure 1. This method of presenting the data has a tendency to straighten the curves somewhat, since there are large individual variations in the slopes of the various segments. No consistent feature has been obliterated, however, or seriously distorted. Individual curves for plasma replenishment almost invariably have an increased slope after one- to two-fifths of the bleeding has been completed. The increase at this time in the apparent rate of plasma replenishment appears to coincide with the steep decline in arterial pressure which has been described in earlier reports (Lawson, 1944). It was particularly noticeable on the final bleeding, as is shown in the figure.

If the rate of dye disappearance into the tissues does not change during the bleeding, these data mean that extravascular fluid is added to the circulation at an increased rate when mean arterial pressure begins to fall as a result of bleeding. Using totally different methods, Adolph, Gerbasi, and Lepore (loc. cit.) found a mean rate of plasma dilution equal to 0.25 cc./kgm./min. following rapid moderate hemorrhage. The mean rate of plsama replenishment in our experiments, on the first bleeding, was only 0.154 ± 0.0114 cc./kgm./min. If our values were larger than theirs, excessive dye disappearance might be suspected. The fact that our values are smaller may be attributed to differences in the type and volume of hemorrhage. If hemorrhage produces changes which result in excessive rates of dye disappearance, the rate of disappearance might be expected to be greater during the second than during the first bleeding. The mean rate of apparent plasma replenishment on the second bleeding was only 0.088 ± 0.0119 cc./kgm./min., which is significantly less than the rate on the first.

In experiments designed for the production of hemorrhagic shock, and employing much slower rates of bleeding than ours, Price, Hanlon, Longmire and Metcalf (loc. cit.) found a mean rate of plasma replenishment equal to about 0.101 cc./kgm. for each cubic centimeter of blood drawn. Their animals were probably, during most of the bleeding, in a state not unlike our animals on the final bleeding. Our rate for the final bleeding, stated in equivalent terms, was 0.088 cc./kgm. for each cubic centimeter of blood drawn, which is in fairly good agreement.

If dye were lost to the tissues at excessive rates during the bleeding, it would presumably have to escape along with the protein to which it is bound (Rawson, loc. cit.). Dye which escaped during the first bleeding would not be expected to return to the circulation after the injection in our experiments since there is only a very slow replenishment of circulating protein (Ebert, Stead, Warren and

Watts, 1942). Excessive dye disappearance during the first bleeding should accordingly leave less dye remaining in the circulation a few hours later than

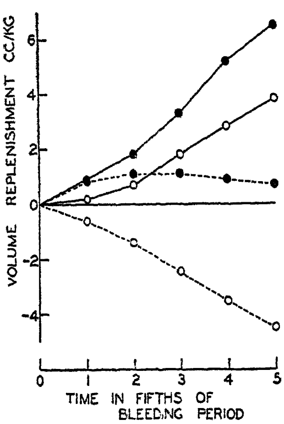

Fig. 1

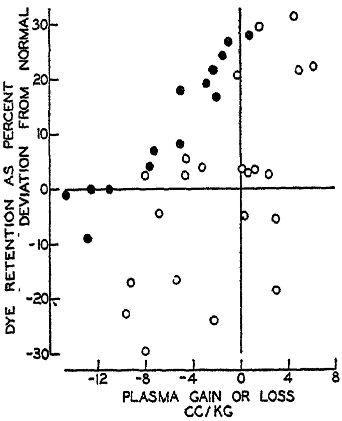

Fig. 2

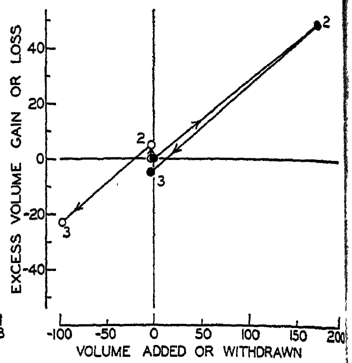

Fig. 3

Fig. 1. The replenishment of plasma and cell volumes during bleeding. The values given are the averages for 15 dogs. Volume replenishment at any instant during the bleeding was obtained as the sum of the volume drawn plus the volume remaining in the circulation, minus the initial circulating volume. An individual curve was constructed for each dog, the total duration was divided into fifths, and the values at these intervals were used for obtaining the averages. The solid lines give plasma replenishment, the broken lines cell replenishment. The solid circles give the values for the first bleeding; the hollow circles values for the final bleeding. Thirty-six cubic centimeters per kilogram of whole blood were given at the end of the first bleeding. The interval between bleedings was approximately four hours.

Fig. 2. The relationship between dye and plasma retention. Plasma loss was calculated as the sum of the volume circulating at the end of the first bleeding plus the volume injected, minus the volume circulating four hours later. Average normal dye retention was calculated from our values for total circulating dye at the end of first bleeding, and the length of the interval, using the formula $D_e\left(1 - \sqrt{\frac{t}{T}}\right)$, where D_e is the amount of dye estimated to remain in the circulation at the end of the first bleeding, t is the time in hours ($3\frac{1}{2}$ to $4\frac{1}{2}$ hrs.), and T has the average value 36.4 found by King, Cole and Oppenheimer in normal dogs (loc. cit.). Total circulating dye at the end of the interval was calculated from the dye concentration and the plasma volume as measured by immediately subsequent new dye injection. Solid circles give values for dogs injected with whole blood at the end of the first bleeding, and open circles values for dogs injected with gelatin solutions.

Fig. 3. The excess gain of cells following injection, and the excess loss during bleeding. Average values are given as solid circles for dogs injected with whole blood (15 animals) and as hollow circles for dogs injected with gelatin solutions (24 animals). Three values are given for each group: 1. The measured circulating cell volume at the end the first bleeding (not numbered in the figure), plotted at 0:0 for the first group and at 0: −2 for the second. Cell volumes at these points are 264 and 262 ccm., respectively. 2. The measured circulating cell volume four hours after the injection. 3. The measured circulating cell volume at death on the final bleeding. At each of these points the measured change in circulating volume is the sum of the changes on both axes. All volumes in cubic centimeters of cells.

in normal dogs. Estimates of the residual circulating dye four hours after the termination of the first bleeding were made in all our experiments. Using the formula which was found by King, Cole and Oppenheimer to give the average rate of dye disappearance in normal undisturbed dogs (1943), we would expect to find remaining in our animals from 64 per cent to 69 per cent of the dye which was circulating at the end of the first bleeding, the percentage depending upon the exact length of the interval. Any large consistent error in our assumptions, or any deviation from the normal in the rate of dye disappearance during the four-hour interval, should cause a deviation in our average value. The average residual dye found at the end of four hours was 3.3 per cent in excess of the expected amount. The difference is not considered significant. There is considerable scatter in the data, which may not be any greater than the scatter observed in normal dogs, since the formula contains a constant whose coefficient of variation is approximately 0.50.

The individual data are given in figure 2 as percentage deviations of the observed values from the expected average, and are plotted against the volume of plasma gained or lost following the injection. Practically all the dogs who received whole blood on the injection appear to have lost plasma. Data are given elsewhere which show that this loss is nearly equal to the plasma volume gained during the bleeding, which suggests that the inflow of fluid during the bleeding is reversed by the injection (Lawson and Rehm, 1945a). Some of the dogs receiving gelatin solutions gained, and others lost plasma volume. These data are discussed elsewhere (Lawson and Rehm, 1945b). It is apparent from the figure that very few animals gained plasma and lost excessive amounts of dye; that most of the dogs injected with whole blood retained more dye than was expected, despite the loss of plasma; and that the greatest dye losses occurred in the gelatin treated dogs. It does not seem profitable to attempt an interpretation of these data without additional information. They are introduced here as contributory, but admittedly inconclusive, evidence against excessively rapid rates of dye disappearance during the bleeding.

Cell volume. The changes in cell volume during the first bleeding are more variable than the changes in plasma volume. The terminal decline shown in the average curves of figure 1 is much more marked in some individual cases, with a slope which is nearly the same as the slope for the final bleeding. Curves for the final bleeding are less variable. In most cases cells appear to be lost throughout the bleeding in excess of the volume withdrawn. The rate of excess cell volume loss is nearly constant during the bleeding, as is shown in the figure.

It is well known that the spleen contracts during hemorrhage, and it is reported to remain contracted in hemorrhagic shock even though blood is given by transfusion (Lewis, Werle and Wiggers, 1943). Withdrawal of circulating cells into the splenic and other cell reservoirs during the final bleeding can therefore be dismissed as improbable. Peripheral systemic trapping of cells could account for the excessive loss, but could hardly produce the nearly linear losses which were observed. Such trapping should be confined to the terminal portion of the bleeding, when the flow has ceased in most systemic capillaries.

The data of figure 3 throw considerable light on the mechanism, and suggest that a systematic error in our measurements of circulating cell volume is responsible for the apparent excessive loss of cells. The figure is constructed to show the measured circulating cell volume at the end of the first bleeding, and its change through the remaining stages of the experiments. No values prior to the end of the first bleeding are given in the figure. When cells were injected (whole blood experiments) the increase in circulating cell volume was 28.5 per cent greater than the volume of cells injected. When cell-free fluids were injected (gelatin experiments) there was no significant change in the measured circulating cell volume. When cells were withdrawn by bleeding, the loss in circulating cell volume in the first group was 30 per cent, and in the second group 29 per cent greater than the volume withdrawn. On the final bleeding the circulating cell volumes in the gelatin-injected dogs were considerably smaller, as is shown in the figure, than in the dogs injected with whole blood. Their average cell volume at death was only 143 cc. as compared with 258 cc. at death in the whole blood experiments. Since the excess cell volume loss, as a percentage of the volume withdrawn, is about the same in the two groups, there is no reason to believe that the percentage changes appreciably with a reduction in the circulating cell volume. If it were possible to maintain the circulation indefinitely by injection of cell-free fluids, it thus appears that the cells would disappear completely from the drawn blood after the volume of cells drawn equaled about 77.5 per cent of the initial measured volume. It is probable that our methods overestimate all circulating cell volumes by an average of about 29 per cent. That is to say, the true volumes are about 77.5 per cent of those measured.

TABLE 1

All values are average cell volumes, in cubic centimeters. The expected terminal volume is the sum of the initial volume plus the volume reinjected, minus the total volume drawn (first and final bleeding). The observed terminal volume is the volume remaining in the cardiovascular system at death. The first group of animals received whole blood, the second gelatin solutions, at the end of the first bleeding.

NO. ANIMALS	INITIAL VOL.	VOL. REINJECTED	TOTAL VOL. DRAWN	EXPECTED TERM. VOL.	OBSERVED TERM. VOL.
15	460	172	379	253	258
24	460	0	323	137	143

This estimate of the magnitude of the error is in good agreement with the estimate made by Stead and Ebert (1942). They bled splenectomized dogs repeatedly and reinjected plasma. The circulating cell volume one or two days later was found by methods similar to ours to be 21 to 34 per cent less than the original volume minus the volume withdrawn. The close agreement between their data and ours suggests that our animals behave like splenectomized dogs after the first bleeding has been completed.

If the measurements which we made at intermediate stages are disregarded,

and the expected terminal cell volume is computed from the initial volume, the volume reinjected, and the volume drawn in the two bleedings, the phenomenon of excessive loss during exsanguination is completely masked. These data are given in table 1. It seems likely that cells are added to the circulation from cell reservoirs during the first bleeding in just about sufficient volume to cover the error. If the error is assumed, on the strength of the data given in figure 3, to be constant at about 29 per cent throughout all stages of exsanguination, the cell reservoirs must have added to the circulation during the first bleeding a volume of cells equal to slightly more than 29 per cent of the volume drawn.

SUMMARY AND CONCLUSIONS

No evidence was obtained for an increase in the rate of disappearance of the dye T-1824 during almost complete exsanguination or in the four-hour period following reinjection of blood, in experiments on barbitalized dogs. Indirect measurement of circulating plasma volume during exsanguination therefore seems legitimate. Such measurements indicated that fluid was added to plasma at the mean rate of 0.154 ± 0.0114 cc./kgm./min. when blood was drawn at the rate of 2 cc./kgm./2 min. until mean arterial pressure fell below 20 mm. Hg. When the animals were kept alive by reinjection of blood, and were bled to death at the same rate four hours later, the rate of plasma replenishment during the second bleeding was only 0.088 ± 0.0119 cc./kgm./min. The rate of addition of fluid to plasma was usually increased at the time when arterial pressure began to decline steeply during the bleeding.

Evidence was obtained that the circulating cell volume is over-estimated by an average of about 29 per cent if it is calculated from the plasma volume and the hematocrit in drawn blood. When measured by this method, the gain in circulating cells following injection was about 29 per cent greater than the volume injected; and the loss during bleeding was about 29 per cent greater than the volume withdrawn. The percentage error seems to remain fairly constant throughout all stages of exsanguination. The error appears in most cases to be completely masked during the first bleeding in our experiments, presumably by expulsion of cells from the cell reservoirs.

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THE REVERSIBILITY OF THE CARDIOVASCULAR DAMAGE DONE BY NEARLY COMPLETE EXSANGUINATION

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It is reasonable to suppose that the volume of blood-loss required to stop the circulation will vary with certain changes in the state of the cardiovascular system. Simms, in 1942, determined the fatal hemorrhage volume in rats, and found that the average volume changed with the age of the animals. Methods have been described by the present authors for measurement of the change in fatal hemorrhage volume in dogs (Lawson, 1943, 1944; Lawson and Rehm, 1943). A control estimate is obtained by extrapolation after incomplete exsanguination. The final measurement is made by bleeding until the circulation stops permanently. Under the conditions of the previous studies, when all or a moderately large part of the drawn blood was reinjected at the end of the control bleeding, the fatal hemorrhage volume a few hours later was found to be equal to the sum of the residual and the reinjected volumes. The empirical data thus provided no evidence that the control bleeding produced any irreversible impairment of the ability to withstand hemorrhage except for the net loss of blood volume incurred in the bleeding and reinjection. In all of these studies the control bleeding was discontinued when mean arterial pressure fell below 60 mm. Hg, or after no more than 10 cc./kgm. had been drawn below 60 mm.

The present studies were undertaken because extrapolation for the residual bleeding volume at 60 mm. Hg was found to be unreliable in periods of very warm weather (unpublished data). This report presents data on animals which were bled during the control observations to considerably lower end-points (below 20 mm. Hg mean arterial pressure), at which the accuracy of extrapolation for the small residual volume does not seem to be subject to seasonal variations. These animals, when reinjected with blood, were consistently found on subsequent bleeding to yield less than the sum of the residual and the reinjected volumes. The general equation for the fatal hemorrhage volume as measured in our experiments is:

$$H = Q + I - L,$$

where H is the volume drawn by controlled arterial hemorrhage before the circulation comes to a standstill (termed the bleeding volume), Q is the blood volume at the start of the hemorrhage, I is the volume gained from extravascular sources during the bleeding, and L is the liminal volume required to keep blood flowing into the arteries (Lawson, 1944). A loss of bleeding volume, as in the present experiments, must accordingly be due to a decline in the values for Q or I in the equation, or to an increase in the value for L. An attempt was made to derive

the values which would be expected for each of these factors if the reinjection of blood produces complete reversal in proportion to the volume injected. These values were compared with the values actually found on the final bleeding.

Derivation of expected values. The values actually measured were:

Q_0 = blood volume at the start of the control observations.

Q_{1e} = blood volume at the end of the control bleeding.

H_{1a} = volume drawn in control bleeding.

R = volume reinjected at end of control bleeding.

Q_2 = blood volume at the start of the final bleeding, about four hours after the reinjection.

H_2 = volume drawn in final bleeding.

L_2 = blood volume at the end of the final bleeding, i.e., when the circulation of blood ceases.

Values which were assumed or calculated were:

H_{1e} = residual bleeding volume at the time the control bleeding is discontinued. Assumed to have a value of 5 cc./kgm. on the strength of data presented below.

I_1 = volume gained from extravascular sources during the control bleeding, calculated as $I_1 = H_{1a} + Q_{1e} - Q_0$.

I_2 = volume gained from extravascular sources during the final bleeding, calculated as $I_2 = H_2 + L_2 - Q_2$.

At the time the first bleeding is discontinued, the small residual bleeding volume is:

$$H_{1e} = Q_{1e} + I_{1e} - L_{1e},$$

where I_{1e} is the additional volume which would be gained from extravascular sources if the bleeding were continued to stoppage of the circulation, and L_{1e} is the blood volume which would be required to maintain a minimal cardiac output at the end of a complete exsanguination. Since any value for I is a function of time, and since the first bleeding is nearly a complete exsanguination in these experiments, I_{1e} must have a very small value unless there is an excessively rapid rate of blood replenishment during the last portion of a complete exsanguination. There is no evidence for a terminal increase in the rate of blood replenishment (Lawson and Rehm, 1945). For the sake of simplicity it is therefore permissible to give I_{1e} a value of O. Furthermore, if the bleeding were continued past the present end-point to stoppage of the circulation, the volume remaining in the cardiovascular system at death would be called L_1 in conformity with the terminology employed. The residual bleeding volume at the end of the first bleeding is accordingly

$$H_{1e} = Q_{1e} - L_1 \qquad \text{(a).}$$

For the derivation of expected values it may be assumed that under ideal conditions the reinjection at this time of a volume R of blood will reverse all the changes which have occurred while the last R volume was being withdrawn. If this were the case, any volume of fluid or cells added to the circulation during

withdrawal of R should leave the circulation after R has been reinjected. If volume replenishment occurs at a constant rate throughout the bleeding, $\frac{I_1R}{H_{1a}}$ must enter the circulation during withdrawal of R, and leave the circulation after its reinjection. This formulation is obviously inexact if the rate of volume replenishment changes during the bleeding, as seems to be the case (Lawson and Rehm, 1945). But so long as the volume R is not very different from the volume H_{1a}, as in the present experiments, the error in the formulation should not be large. It may be expected, therefore, that a steady state will be reached some time after the reinjection, with a circulating blood volume equal approximately to $Q_{1e} + R - \frac{I_1R}{H_{1a}}$.

If the animal were now completely exsanguinated, the volume added during the bleeding should again be equal to $\frac{I_1R}{H_{1a}}$, and the volume remaining in the cardiovascular system at death should have its original value L_1. From equation *a:*

$$L_1 = Q_{1e} - H_{1e} \tag{b}$$

The equation for the final bleeding volume under these ideal conditions should therefore be:

$$H_h = \left(Q_{1e} + R - \frac{I_1R}{H_{1a}}\right) + \frac{I_1R}{H_{1a}} - (Q_{1e} - H_{1e}) \tag{c}$$

$$= H_{1e} + R \tag{d}$$

In the actual experiments the observed final bleeding volume is

$$H_2 = Q_2 + I_2 - L_2 \tag{e}$$

The present study undertakes to determine whether the deviations of H_2 from its expected ideal value as given in equation *d* are due to deviations in Q_2, I_2, or L_2 from their ideal values as formulated in equation *c*.

METHODS. Normal mature street dogs were deprived of food and water for approximately 24 hours before beginning the experiments. They were allowed moist food but no water on the last feeding, and were accordingly probably somewhat more dehydrated than the animals of the previous studies. They were anesthetized by a single intravenous injection of 250 mgm./kgm. of sodium barbital at least three hours before starting the observations. Some of the animals, designated as saline-pretreated, received 15 cc./kgm. of 0.9 per cent NaCl solution intravenously immediately after the barbital injection.

Blood was drawn from a femoral artery by free bleeding in unit volumes of 2 cc./kgm., repeated at intervals of two minutes. Mean arterial pressure was recorded from a mercury manometer connected with the other femoral artery. A straight manometer was used to facilitate the accurate reading of low pressures. To ensure a standard procedure, bleeding was continued during the first observations until pressure remained below 20 mm. Hg for an entire two minute period.

In the experiments reported here, 36 cc./kgm. of heparinized autogenous blood were then reinjected, the injection being started immediately and completed within four to eight minutes. When less than this volume was available for injection, autogenous blood was supplemented with freshly drawn donor blood.

Respiration was recorded from a sternal pneumograph in order to detect premature cessation of breathing. Animals whose breathing ceased more than two minutes before the 20 mm. endpoint was reached usually either died or had very low bleeding volumes after the reinjection. Lesser degrees of premature respiratory failure appeared to be without effect on the subsequent course of the experiments. Premature failure of breathing was observed more frequently in periods of very warm weather. Manual artificial respiration was given after the beginning of the reinjection if spontaneous breathing did not start within one minute. Artificial respiration was never employed before starting the reinjection on the first observations, or at any time during the final bleeding.

The final bleeding was done in the same manner as the first. It was begun approximately four hours after the reinjection, and was continued until mean arterial pressure remained permanently below 10 mm. Hg, an endpoint which nearly coincides with disappearance of the arterial pulse. As a rule breathing ceased on the final bleeding while mean arterial pressure was above 20 mm. Hg.

Direct measurements of plasma volume were made at the beginning of both the control and the final bleeding, by a modification of the method of Gibson and Evans (1937). Indirect measurements were made at intervals during both bleedings and at their termination. Circulating cell volumes were calculated from the plasma volumes and the cell:plasma ratios in drawn blood. A detailed description of the methods used, and a critical examination of their validity, have been given elsewhere. The data on plasma volume are probably reasonably reliable. The values for circulating cell volume are probably something like 29 per cent too high (Lawson and Rehm, 1945).

RESULTS. *The residual bleeding volume at 20 mm. Hg mean arterial pressure.* Forty-six dogs have been bled to death at the present hemorrhage rates on the first observation. The average volume drawn below 20 mm. Hg was 4.96 cc./kgm., with a standard deviation of 2.22 cc./kgm. Twenty-eight of these were dehydrated for 24 hours; 18 were saline-pretreated. The means with their standard errors for the two groups were 5.14 ± 0.554 and 4.66 ± 0.397 cc./kgm. respectively. It thus appears that the bleeding volume below 20 mm. is relatively uninfluenced by the state of hydration. A similar comparison of summer and winter animals fails to reveal any significant difference. The data have been gathered over a period of two years, and probably contain adequate samples of seasonal and climatic variations. In rare instances a single unit withdrawal at a time when pressure was still above 20 mm. caused a precipitous fall in pressure with stoppage of the circulation. Such animals were not used in the experiments. It is accordingly known that at least one more unit withdrawal of 2 cc./kgm. would be required to stop the circulation in all the experiments. At 0.05 probability, therefore, the residual bleeding volume at 20 mm. Hg lies between 2 and 9.40 cc./kgm. The value used in the calculations (H_{1e}) was 5 cc./kgm.

Figure 1 shows the relationship between the circulating blood volume at 20 mm. and the initial circulating volume. It is apparent from the figure that the relative invariability of the residual bleeding volume at 20 mm. is not due to any lack of variation in the blood volume remaining in the circulation at this level. The liminal volumes required for the circulation, i.e., the volumes remaining at death, may be approximated by subtracting 5 cc. from the 20 mm. volumes given in the figure. In this form the figure shows the relationship between the liminal blood volume and the volume maintained by the intact animal. The figure suggests that there is a nearly straight-line relationship between the two volumes. A corollary to this is the observation that the volume of blood obtained in the first bleeding has no fixed relation to the initial blood volume, since animals with

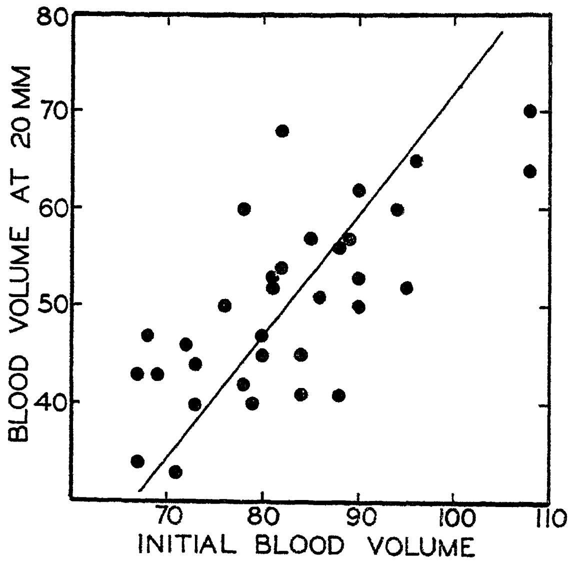

Fig. 1. Blood volumes are the sum of plasma and cell volumes, and are given as cc./kgm. body weight. The equation for the line is: $Q_{20} = 1.261\ (Q_0 - 42.80)$ where Q_{20} is the circulating blood volume when mean arterial pressure has been lowered to 20 mm. Hg by this type of bleeding, and Q_0 is the initial blood volume. $Q_{20} = Q_{1d}$ in all the formulations of this report.

large initial blood volumes are likely to have large liminal volumes. There seems to be a similar straight-line relationship, with less scatter in the data, between initial and terminal plasma volume.

The apparent gain of plasma and cells during the bleeding. These data are given in detail elsewhere (Lawson and Rehm, 1945). They are summarized in the form of averages in table 1 A. Only about half as much plasma replenishment was measured on the final bleeding as on the first. This cannot be ascribed entirely to the shorter duration of the final bleeding. The average rate of plasma gain fell from 0.144 cc./kgm./min. during the first bleeding to 0.073 cc./kgm./min. during the final bleeding in the dehydrated group, and from 0.165 to 0.103 cc./kgm./min. in the pretreated group.

The apparent excessive loss of cells on the final bleeding is attributed to the error in estimating circulating cell volumes by our methods, which was mentioned above. If we are correct in our estimate of the magnitude of the error, the volume of cells added to the circulation from cell reservoirs during the first bleeding is considerably greater than the data of the table indicate. Taking 22 cc./kgm. as the average volume of cells drawn in the first bleeding, approximately 6.4 cc./kgm. of cells must have been added during the bleeding to mask the error. The total cell volumes added during the first bleeding are therefore probably about 6.4 cc./kgm. greater than the volumes shown in the table.

The liminal circulating blood volume. The data are given as averages for the two groups in table 1 B. The liminal volume for both plasma and cells appears to be increased on the final bleeding in the dehydrated group, and to be relatively unchanged in the pretreated group. The increase in the dehydrated group is

TABLE 1

Section A gives the volumes of plasma and cells which appear to be added to the circulation during the bleedings. These volumes are calculated by subtracting the starting volume from the sum of the volume drawn and the volume remaining at the end of the bleeding. Section B gives the liminal volumes of plasma and cells required for the circulation. On the first bleeding these are estimated as 2.5 cc./kgm. less than the volumes found when the bleeding was discontinued. On the final bleeding they are the volumes found just before the circulation ceased. The values given are the averages for seven dehydrated and seven saline-pretreated animals, in cubic centimeters per kilogram body weight.

CONDITION	FIRST BLEEDING			SECOND BLEEDING		
	Plasma	Cells	Total	Plasma	Cells	Total
A. Volume added during hemorrhage						
Dehydrated	5.46	1.54	7.00	2.41	−4.29	−1.88
Pretreated	7.20	−0.84	6.36	3.64	−5.17	−1.53
B. Liminal volume						
Dehydrated	27.40	21.31	48.71	30.80	27.69	58.49
Pretreated	33.97	19.49	53.46	32.83	19.87	52.70

probably somewhat less than these data indicate, as a result of the error in estimating circulating cell volumes. The measured increase is much too large to be attributed to the error alone, however, as is shown in table 3, part B.

The volume of circulating blood after the first bleeding and replacement. The data are given as individual values in table 2. In general the plasma volumes are in fairly good agreement with their expected ideal values. There does not appear from the table to be a significant difference between the pretreated and the dehydrated dogs in this respect. Cell volumes are as a rule larger than the expected values. This could be due to the error in estimating circulating cell volumes, to a failure of the cell reservoirs to remove cells after the injection in the same volume as were added during the bleeding, or even to continued expulsion of cells from the reservoirs after the injection. The values which appear in the last two columns of the table were obtained by calculating the true circu-

lating cell volumes as 77.5 per cent of the measured volumes to cover the average suspected error, and by computing the expected cell volumes simply as the sum of the residual and the reinjected cell volumes. The observed and the expected values, when so derived, are in fairly good agreement. We suspect, for this reason, that reliable measurements of circulating cell volume would show the

TABLE 2

Q_{1e} is the volume remaining in the circulation at the end of the first bleeding. R is the volume injected at the end of the first bleeding. Q_h is the expected volume after attainment of a steady state following the injection. It is computed from the equation $Q_h = Q_{1e} + R - \frac{I_1R}{H_{1a}}$, where I_1 is the total volume replenishment during the first bleeding, and H_{1a} is the volume drawn on the first bleeding. Q_2 is the observed circulating volume approximately four hours after the injection. Q'_h is the expected cell volume computed as 0.775 $Q_{1e} + R$, and Q'_2 is 0.775 × the observed cell volume. All volumes as cubic centimeters per kilogram of body weight.

DOG NO.	PLASMA VOLUME					CELL VOLUME					CELL VOLUME REVISED	
	Q_{1e}	R	$\frac{I_1R}{H_{1a}}$	Q_h	Q_2	Q_{1e}	R	$\frac{I_1R}{H_{1a}}$	Q_h	Q_2	Q_h'	Q_2'
Dehydrated												
1	22.0	19.0	3.5	37.5	38.2	18.2	17.0	2.0	33.2	40.0	31.1	31.0
2	39.7	19.3	7.8	51.2	41.4	42.5	16.7	17.6	41.6	57.7	49.6	44.7
3	27.4	18.4	3.6	42.2	44.8	24.4	17.6	−1.6	43.6	48.6	36.5	37.7
4	31.3	17.6	4.2	44.7	47.5	32.7	18.4	0.0	51.1	58.6	43.7	45.4
5	32.3	20.3	5.8	46.8	40.2	21.5	15.7	−0.8	38.0	49.2	32.4	38.1
6	26.9	21.1	5.9	42.1	40.3	18.7	14.9	2.1	31.5	37.2	29.4	28.8
7	38.0	21.5	5.6	53.9	61.5	21.9	14.5	−1.3	37.7	45.4	31.5	35.1
Average				45.49	44.84				39.53	48.10	36.31	37.26
Pretreated												
8	44.6	19.7	7.2	57.1	56.7	31.0	16.3	−3.4	50.7	54.0	40.3	41.8
9	32.1	19.7	5.1	46.7	45.8	23.0	16.3	0.7	38.6	42.3	34.1	32.8
10	25.1	19.9	4.6	40.4	45.9	17.2	16.1	0.0	33.3	39.5	29.4	30.6
11	29.6	19.7	4.8	44.5	47.1	23.0	16.3	4.4	34.9	43.7	34.1	33.9
12	44.0	24.1	7.8	60.3	55.4	19.0	11.9	−1.3	32.2	31.7	26.6	24.6
13	45.8	23.4	4.5	64.7	54.6	18.7	12.6	−6.0	37.3	33.7	27.1	26.1
14	34.1	20.8	6.2	48.7	45.0	21.0	15.2	−1.4	37.6	33.0	31.5	25.6
Average				51.77	50.07				37.80	39.70	31.87	30.77

circulating volume four hours after the injection to be nearly equal to the sum of the residual and the reinjected volumes. This would mean that expulsion of cells from the reservoirs is complete by the time the first bleeding is finished, and that the reservoirs are not refilled with cells following the injection.

The bleeding volume after exsanguination and replacement. The final bleeding volumes are given in table 3, part A, in the form of averages. Included in the

table are some animals for whom no data are available except the empirical values for bleeding volume. The average final bleeding volume for the 14 dehydrated dogs was 31.3 ± 1.62 cc./kgm. The average for the 18 pretreated dogs was 36.6 ± 0.78 cc./kgm. The expected value is approximately 41 cc./kgm. in both groups ($H_{1e} + R = 36 + 5$). The average loss of bleeding volume after the reinjection is thus 5.3 cc./kgm. greater in the dehydrated than in the pretreated group. An equation for gain of bleeding volume, in which a negative

TABLE 3

All values are average whole blood volumes as cc./kgm., obtained as the sum of plasma and cell volumes. H_{1a} is the volume drawn on the first bleeding. H_2 is the volume drawn on the final bleeding. $Q_2 - Q_h$ is the difference between the observed and the expected circulating volume at the start of the final bleeding, obtained from table 2. $L_2 - L_h$ is the difference between the observed and the expected liminal volume on the final bleeding, obtained from table 1 B by taking L_h equal to the liminal volume on the first exsanguination. $H_2 - H_h$ is the difference between the observed and the expected final bleeding volume, obtained by taking H_h equal to 41 cc./kgm. $I_2 - I_h$ is the difference between the observed and the expected volume replenishment on the final bleeding. I_2 is given in table 1 A, under the second bleeding. I_h is given in table 2 as $\frac{I_1R}{H_{1a}}$. Part A gives unrevised values. In part B, circulating cell volumes were calculated as 77.5 per cent of the observed values, and the expected cell volume at the start of the final bleeding was computed as the residual plus the reinjected volume. Groups marked with an asterisk appear in both parts of the table.

NO. DOGS	CONDITION	H_{1a}	H_2	$Q_2 - Q_h$	$I_2 - I_h$	$L_2 - L_h$	$H_2 - H_h$
Part A							
7*	Dehydrated	40.0	32.59	7.92	−6.55	9.78	−8.41
7	Dehydrated	46.9	30.00				−11.00
7*	Pretreated	45.4	35.69	0.20	−6.27	−0.76	− 5.31
11	Pretreated	54.5	37.27				− 3.73
Part B							
7*	Dehydrated	40.0	32.59	0.94 ±1.266	−3.12 ±4.080	6.23 ±4.445	−8.41
7*	Pretreated	45.4	35.69	−2.80 ±2.504	−3.05 ±0.554	−0.54 ±2.795	− 5.31

value means a loss, can be obtained as the difference between the equation for observed and the equation for expected bleeding volume (equation e — equation c as given in the introduction). The equation may be written in the form:

$$H_2 - H_h = (Q_2 - Q_h) + (I_2 - I_h) - (L_2 - L_h),$$

where subscript *2* denotes the observed, and subscript h the expected values; and where H is bleeding volume, Q is blood volume at the start of the bleeding,

I is volume replenishment during the bleeding, and L is the liminal circulating volume. The data in the last four columns of table 3 are given in the form required for this equation. The values in part A are unrevised volumes. Those in part B are corrected for the suspected error in the measurement of circulating cell volumes, and include the modification of the expected cell volumes suggested in the foregoing section. Essentially the same conclusions may be drawn from either set of data. The loss of bleeding volume in the dehydrated dogs can be attributed entirely to an elevation of their liminal circulating volumes, positive and negative deviations in the two remaining factors in the equation nearly canceling out. An elevation of the liminal volume does not occur in the pretreated dogs. The small loss of bleeding volume which was observed appears

TABLE 4

The end-point is the level of mean arterial pressure, in mm. Hg, at which the first bleeding was terminated. H_{1e} is the residual bleeding volume assumed to be unwithdrawn at the end-point, on the basis of control studies. R is the volume reinjected. H_h is the expected final bleeding volume approximately four hours after the injection, calculated as $H_{1e} + R$. H_2 is the observed final bleeding volume. All volumes are average whole blood volumes, and are given as cubic centimeters per kilogram of body weight. Means for H_2 are given with S.E.

NO. DOGS	CONDITION	END-POINT	H_{1e}	R	H_h	H_2	$H_2 - H_h$
		mm.					
14*	Dehydrated	60	22.5	20	42.5	40.53 ±0.69	−1.97
14	Dehydrated	20	5.0	36	41.0	31.3 ±1.62	−9.70
7	Pretreated	60	22.5	20	42.5	36.9 ±1.50	−5.6
18	Pretreated	20	5.0	36	41.0	36.6 ±0.78	−4.4

* Lawson, 1944.

to be due to a moderate loss of blood volume after the reinjection, and to a reduction in the ability to replenish volume during hemorrhage.

Table 4 summarizes the available empirical data on the loss of bleeding volume after partial exsanguination to two different endpoints, and reinjection of blood. The volume of blood which can be drawn after mean arterial pressure has fallen to 60 mm. Hg does not appear to be significantly different in pretreated and in dehydrated dogs (unpublished data). The residual bleeding volume was accordingly taken to be 22.5 cc./kgm. in the pretreated as well as in the dehydrated animals which were reinjected at this endpoint (Lawson, 1944). In pretreated animals the loss of bleeding volume appears from the table to be about the same for the two endpoints. The data thus offer no evidence that continued bleeding to lower pressure levels does additional damage in pretreated dogs. It is possible

that the first bleeding is in no way responsible for the bleeding volume losses observed in these animals, and that all the changes noted in table 3 would occur with time alone.

In contrast, the dehydrated dogs appear from the table to suffer significantly greater losses of bleeding volume after replacement at 20 mm. than after replacement at 60 mm. The excessive losses in this group must result from changes occurring during the terminal portion of the first bleeding. It has already been shown that as much as 10 cc./kgm. can be drawn below the 60 mm. endpoint without affecting the subsequent loss of bleeding volume (Lawson, 1944).

Discussion. If the data on circulating cell volumes are thrown out on grounds of unreliability, it becomes, of course, impossible to construct complete equations for bleeding volume. The conclusions which may be drawn from the remaining data on plasma volumes, however, are not materially different from those which are drawn from the data on whole blood volumes. The rate of plasma replenishment is less during the second bleeding than during the first. This could mean depletion of extravascular fluids by the first bleeding, or impairment of vasomotor mechanisms which are important in their mobilization. The liminal plasma volume required for the circulation of blood is elevated in the dehydrated but not in the pretreated animals following the first bleeding and reinjection. This value should depend upon the state of vasomotor mechanisms which are responsible for cardiac filling, as well as upon the state of the heart itself. A heart which has been damaged in certain ways may be expected to fail to deliver blood to the arteries while there is still a comparatively large volume being returned to it. Similarly, failure of peripheral mechanisms which aid in returning blood to the heart should produce circulatory failure while the volume in the vascular system is still relatively large. The elevated liminal volumes in the dehydrated dogs suggest that one or both types of damage have been produced. The prevention of this type of damage by saline pretreatment is in conformity with a large amount of clinical and experimental data. Plasma volumes four hours after the reinjection are usually less than the sum of the residual and the reinjected volumes. The volume of plasma which disappears from the circulation after the reinjection appears to be very nearly equal to the volume which enters during the bleeding. If the fluid which enters during the bleeding is protein-free, and if it is brought in only by the lowering of capillary filtration pressure, it would be expected to leave the circulation after the reinjection, when capillary pressure is restored. There was no consistent loss of plasma volume in excess of the amounts expected from these causes. There is thus no evidence from our experiments that endothelial damage is done in the first bleeding such as to permit excessive protein leakage. Such damage may, of course, have occurred in the occasional animals which died following the first bleeding (one in the present experiments).

The cell-volume data are included for the purpose of obtaining a more complete comparison of the dehydrated and the pretreated animals. We are not prepared to suggest that true values for cell volumes can be obtained by applying average corrections to all the data as in tables 2 and 3. The values obtained in

this way seem more reasonable, and are probably somewhat closer to the true values, on the average, than the unrevised data. In order to use more reliable methods for the measurement of cell volume during and at the end of the bleedings, we would have been forced to modify considerably our bleeding and reinjection routines.

SUMMARY AND CONCLUSIONS

Barbitalized moderately dehydrated dogs were almost completely exsanguinated by controlled bleeding in order to determine the liminal circulating blood volume. There appears to be a nearly straight-line relationship between the circulating blood volume in the intact un-bled animal, and the liminal circulating volume. When the animals were kept alive by reinjection of blood, the liminal circulating volume as determined by a second, complete exsanguination, was usually considerably elevated. The circulating plasma volume at the start of the final bleeding was usually less than the sum of the volume remaining at the end of the first plus the volume reinjected, by an amount which is nearly equal to the volume of extravascular fluid added during the first bleeding. It thus appears that nearly all the fluid which enters the circulation during the first bleeding leaves the circulation after the reinjection. If allowance was made for a systematic error in the measurement of circulating cell volumes, the cell volume at the beginning of the final bleeding appeared to be nearly equal to the sum of the residual and the reinjected volumes. No reinjected cells thus appear to be withdrawn from the circulation. The average volume of blood drawn on the final bleeding was 9.7 cc./kgm. less than the sum of the residual bleeding volume, as estimated from control studies, and the volume reinjected. The elevation of the liminal circulating volume is just about enough to account for this loss of bleeding volume.

In similar experiments on dogs pretreated by intravenous injection of 15 cc./kgm. of 0.9 per cent NaCl solution, there was no elevation of the liminal circulating volume on the final bleeding. The average volume of blood drawn on the final bleeding was only 4.4 cc./kgm. less than the sum of the residual bleeding volume and the volume reinjected. This moderate loss of bleeding volume following the reinjection appears to be due to a decline in circulating blood volume during the interval, and to a reduction in ability to replenish volume on the final bleeding. These changes cannot be attributed with assurance to any damaging effects of the first exsanguination, since the loss of bleeding volume in pretreated dogs is equally great if the first bleeding is terminated long before the exsanguination is complete. In dehydrated dogs the loss of bleeding volume is much less when the first bleeding is terminated earlier.

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THE EFFICACY OF GELATIN SOLUTIONS AND OTHER CELL-FREE FLUIDS IN REVERSING THE EFFECTS OF NEARLY COMPLETE EXSANGUINATION[1]

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Moderately dehydrated dogs have been shown to undergo irreversible changes during severe controlled hemorrhage, such that, even though the drawn blood is reinjected, their ability to withstand subsequent bleeding is impaired (Lawson and Rehm, 1945a). The fatal hemorrhage volume (bleeding volume) a few hours later is usually less than the volume of blood reinjected. It is always less than the reinjected volume plus the residual bleeding volume which the animal had at the termination of the first hemorrhage. The factors which determine bleeding volume are: *a*, the circulating volume at the start of the bleeding; *b*, the volume which is added to the circulation during the bleeding, and *c*, the liminal circulating volume. The reduction in bleeding volume under these experimental conditions appears to be due largely to an elevation of the liminal volume which is required to maintain the circulation. These changes may be prevented or greatly reduced by injecting isotonic sodium chloride solution intravenously a few hours before starting the experiments.

The present study shows the degree of reversal which is obtained when plasma, serum, certain gelatin solutions, and 0.9 per cent NaCl solution are given at the end of the first bleeding in place of the drawn blood. A comparative study of this sort has already been reported (Lawson and Rehm, 1943a). The type of hemorrhage employed, and the volumes of replacement were quite different from those of the present studies. The relative efficacy of the various replacement fluids as determined under the present conditions is not identical with that of the former report.

It is obvious that a foreign colloidal solution, when used for replacement in experiments of this type, may maintain for the next several hours a larger circulating volume than whole blood, provided its effective osmotic pressure is greater than that of blood. Under these conditions harmful effects of the foreign colloid could produce considerable elevation of the liminal circulating volume, or marked impairment of the volume replenishing mechanisms which operate during hemorrhage, without causing an excessive reduction in the ability to withstand hemorrhage. In the studies on gelatin solutions, therefore, an attempt was made to obtain, in addition to the empirical data on bleeding volume, data on each of these limiting factors.

Methods. Barbitalized dogs, which had been deprived of water for 24 hours, were bled by controlled arterial hemorrhage at the rate of 2 cc./kgm. every

[1] Aided by a grant from the Knox Gelatine Company, Camden, N. J.

two minutes until mean arterial pressure fell below 20 mm. Hg. Details of the method have been given elsewhere (Lawson and Rehm, 1945a). The residual bleeding volume at this level of exsanguination, as determined by control studies, has been shown to be relatively constant, with an average value in the neighborhood of 5 cc./kgm. Injection of the replacement fluid was started immediately, and was completed within four to eight minutes. Serum and heparinized plasma were freshly prepared from the pooled blood of three to six donor dogs. Since, in confirmation of the earlier study, no difference was observed between serum and plasma, the data for the two are summarized together. Three grades of gelatin were studied, all of which were prepared from a similar beef ossein precursor. Gelation P-20 is the least degraded (largest average molecular weight), L-80 is an intermediate grade, and B-20610-51[2] is so far degraded that 5 per cent solutions remain fluid at ordinary room temperatures. A more complete characterization of these gelatins can be found elsewhere (Cohn, Scatchard and others, 1944).

The final bleeding was begun approximately four hours after the injection of the replacement fluid, and was continued, at the same rate as the first, until mean arterial pressure remained permanently below 10 mm. Hg.

No animals were excluded from the comparative data on bleeding volume except for premature respiratory failure on the first bleeding (see preceding report), or in cases where the drawn volume on the first bleeding was less than 30 cc./kgm. Animals which died within four hours of the replacement were recorded as having a final bleeding volume equal to 0. They obviously could not be included in the study on limiting factors. The comparative data presented in the latter were accordingly obtained on a selected group of animals, and are of limited usefulness in judging the relative efficacy of the fluids.

To obtain data on the limiting factors, plasma and cell volumes were measured at the beginning and end of both bleedings. The methods which were used have been described in detail elsewhere (Lawson and Rehm, 1945b). Expected values for circulating volume after the replacement, volume replenishment during the final bleeding, and the final liminal circulating volume were derived from formulae based on the assumption of complete reversal (Lawson and Rehm, 1945a). All circulating cell volumes were corrected for a systematic error in our methods of measurement.

Empirical data on bleeding volume were obtained in both dehydrated dogs and dogs pretreated by intravenous injection of 15 cc./kgm. of 0.9 per cent NaCl three hours or more in advance of the first bleeding. Data on the limiting factors for bleeding volume were obtained only in dehydrated dogs.

RESULTS. The empirical data on bleeding volume are summarized in table 1. If each group in the table is assumed to have, at the time the first bleeding is terminated, an average residual bleeding volume of 5 cc./kgm., and if injection of the replacement fluid effects complete reversal, the final bleeding volume for each group should be equal to the volume of the replacement plus 5 cc. Thus, the expected bleeding volume is 41 cc. when the volume of replacement is 36 cc.,

[2] Gelatin solutions P-20 and L-80 were supplied by the Knox Gelatine Company, Camden, N. J., and gelatin solution B-20610-51 by the Upjohn Company, Kalamazoo, Mich.

and 31 cc. when the volume of replacement is 26 cc. The last column in the table gives the loss of bleeding volume following the replacement, as the difference between the volume expected on this basis and the volume actually obtained.

When comparable data are available, it is apparent from the table that bleeding volume loss is greater in the dehydrated than in the pretreated dogs, for any given type of replacement. In pretreated animals the iso-osmotic fluids have the following relative efficacy when given in a volume of 36 cc./kgm.: blood > P-20 > plasma and serum > L-80 > B-20610-51 > NaCl. The observed differences in bleeding volume for this series are statistically significant except for the difference between P-20 and plasma-serum. No data were obtained on dogs which had been bled to the 20 mm. endpoint and left without replacement. It has already been shown that dehydrated dogs usually die within two to three hours if they are bled as much as 10 cc./kgm. below 60 mm., and left untreated (Lawson, 1944). It seemed certain that they would die if they were bled still further, to the present endpoint, and left without treatment. Six pretreated dogs were bled 10 cc./kgm. below 60 mm. and allowed to remain unreplaced. All of them died within three hours.

Not all the fluids were studied in both dehydrated and pretreated animals. As a rule, the final bleeding volumes were more variable in dehydrated than in pretreated dogs, as is shown by the standard errors in table 1. Pretreated dogs were used for the more extended comparison. The three iso-osmotic fluids which were studied in dehydrated animals, blood, gelatin P-20 and gelatin L-80, appear from the table to have the same relative efficacy as in pretreated animals, so that a complete comparison did not seem to be necessary. It seemed not unlikely that the values obtained with salt solution would depend largely upon the degree of dehydration. A rigorous comparison of the efficacy of salt solution in dehydrated and pretreated animals might therefore be of some interest. This did not seem necessary for the present purposes, however, since dehydrated dogs which were replaced with 0.9 per cent NaCl after being bled only 10 cc./kgm. below 60 mm., had an average subsequent bleeding volume loss of 18.1 $\pm$ 1.526 cc./kgm. If they were bled to 20 mm. and replaced with salt solution, their loss of bleeding volume would undoubtedly be greater.

Table 2 shows the factors which are responsible for the loss of bleeding volume in dehydrated dogs after replacement with gelatin solutions. Similar data for whole blood replacement, taken from the preceding report, are included to facilitate comparison. With all the gelatin solutions, the circulating plasma volume following the injection was larger than the volume expected. Plasma retention appears from the table to be greater with the hyperosmotic than with iso-osmotic solutions. The average retention of plasma for the gelatin experiments as a whole is greater than that for whole blood experiments, although the difference is not large enough to be unquestionably significant. Circulating cell volumes following gelatin injection were nearly equal to the volumes remaining in the circulation at the end of the first bleeding. If corrected cell values are used, as in the table, there seems to be no difference in this respect between replacement with blood and replacement with gelatin solutions.

The amount of change in the liminal circulating volume in these experiments

was quite variable. In the experiments with blood the standard deviation from the mean change was 11.78 cc./kgm., and in the experiments with gelatin 8.58 cc./kgm. The observed differences in the means are not significant, and it appears that the differences would be of a low order of significance even if much larger groups of animals were used. Volume replenishment during the final bleeding appears from table 2 to be at least as great in the gelatin replaced as in the blood replaced animals.

TABLE 1

Replacement materials P-20, L-80, and B-20610-51 are gelatins of decreasing average molecular size. Percentage solutions marked with an asterisk have approximately the same colloid osmotic pressure *in vitro* as dog plasma. The second column gives the number of dogs which died during the four hour interval following replacement. Their final bleeding volumes were recorded as 0. The final bleeding volumes are given as the means with their standard errors. Bleeding volume deficits are the differences between the expected and the observed final bleeding volumes. A negative value means a gain in bleeding volume following the replacement.

NO. DOGS	DIED	CONDITION	REPLACEMENT			FINAL BLEEDING VOLUME CC./KGM.	BLEEDING VOLUME DEFICIT CC./KGM.
			Material	Vol. cc./kgm.	Conc. gm./100		
13	0	Dehydrated	P-20	36	3.9*	27.38 ± 1.702	13.62
14	0	Dehydrated	P-20	36	6.0	31.14 ± 2.290	8.86
5	1	Dehydrated	P-20	26	3.9*	18.80 ± 4.877	12.20
6	0	Dehydrated	P-20	26	6.0	31.30 ± 5.695	−0.30
8	0	Pretreated	P-20	36	3.9*	31.18 ± 1.261	9.82
3	2	Dehydrated	L-80	36	3.45*	8.70 ± 8.680	32.30
13	0	Pretreated	L-80	36	3.45*	24.77 ± 1.448	16.23
7	0	Pretreated	B-20610-51	36	2.8*	18.00 ± 2.308	23.00
17	9	Pretreated	NaCl	36	0.9	8.71 ± 2.428	32.29
11	0	Pretreated	Plasma, serum	36		29.82 ± 1.218	11.18
14	0	Dehydrated	Blood	36		31.30 ± 1.620	9.70
18	0	Pretreated	Blood	36		36.60 ± 0.780	4.40

Although the change in the total liminal circulating volume does not appear to be significantly different following the two types of replacement, there are significant differences in the liminal plasma volume and in the liminal cell volume. Comparative data are given in table 3. The gelatin treated dogs had a marked elevation of the circulating plasma volume, and a consistent depression of the circulating cell volume, in comparison with the dogs replaced with whole blood. From the difference in magnitude of these changes in the two groups, it is apparent that the gelatin treated animals maintained a minimal circulation with about 8 cc./kgm. less circulating cell volume, and about 11 cc./kgm. more plasma volume, than the dogs replaced with whole blood. These data suggest that

gelatin-plasma and cells are nearly interchangeable, volume for volume, in maintaining the circulation during acute hemorrhage.

DISCUSSION. When these studies were undertaken it was hoped that certain of the limiting factors for bleeding volume would be subject to less individual variation than bleeding volume itself. From the data which have been presented, however, it appears that even larger groups of animals are required to obtain reliable mean values for any one of the limiting factors, than for bleeding volume. Following hemorrhage and replacement, both the starting blood volume and the liminal circulating volume may be considerably elevated or depressed, with no large change in bleeding volume. Because of these unexpectedly large variations, definitive comparative studies on the limiting factors for bleeding volume will require larger groups of animals than we have employed.

TABLE 2

All volumes are averages, and are given as cc./kgm. Expected values were formulated on the assumption of complete reversal. The last four columns give the deficit in bleeding volume as observed on the final bleeding, under the heading ΔH; and the amount contributed to the deficit by the factors: ΔQ = deficit in blood volume at the start of the bleeding; ΔI = deficit in blood replenishment during the bleeding; ΔL = elevation of liminal circulating volume. A minus sign denotes an excess. Deficits were obtained as the differences between expected and observed volumes. All animals had been deprived of water for 24 hours. Summarized mean values for ΔQ, ΔI, and ΔL are given with their standard errors.

NO. DOGS	REPLACEMENT	BLOOD VOLUME AFTER REPLACEMENT				BLEEDING VOLUME DEFICIT			
		Plasma		Cells		ΔQ	ΔI	ΔL	ΔH
		exp.	obs.	exp.	obs				
	Gelatin P-20								
5	36 cc. 3.9%	61.65	62.58	15.02	14.50	−0.58	1.78	8.62	9.82
6	36 cc. 6.0%	58.17	63.52	14.60	15.72	−6.63	1.58	12.00	6.95
4	26 cc. 3.9%	50.60	54.30	17.58	17.82	−3.94	2.68	8.78	7.52
6	26 cc. 6.0%	52.87	57.78	16.78	16.87	−5.00	−0.18	4.88	−0.30
21	*Ave. gelatin P-20*	*55.98*	*59.96*	*15.91*	*16.14*	*−4.21* ±1.18	*1.44* ±1.69	*8.58* ±1.86	*5.81*
7	*Ave. 36 cc. blood*	*45.49*	*44.84*	*36.31*	*37.26*	*−0.94* ±1.27	*3.12* ±4.08	*6.23* ±4.45	*8.41*

The elevation of the liminal circulating volume observed in dehydrated dogs after the first bleeding, in experiments with whole blood replacement, is an index to the amount of unreversed damage done to the cardiovascular system during the first bleeding. If the replacement fluid used as a substitute for blood is inadequate in any particular, or if it produces additional damaging effects, an even greater elevation of the liminal circulating volume should occur. Although our studies are incomplete, the data are sufficient to show that there is not much difference in the amount of elevation obtained with blood and with gelatin solutions. This is an unexpected finding, since any cell-free fluid, such as plasma,

would be expected on *a priori* grounds to produce a greater elevation of the liminal volume than is produced by whole blood. The adequacy of gelatin solution as a blood substitute is also shown by the comparative data on blood replenishment during the final bleeding. It is evident that the mechanisms responsible for blood replenishment during hemorrhage function at least as well in the presence of a mixture of gelatin and blood, as in the presence of blood alone.

The empirical data on bleeding volume in pretreated animals show that reversal is considerably more complete with whole blood than with any of the cell-free fluids. Until comparative studies on the limiting factors are complete, no interpretation can be attempted. Under the former experimental conditions no such difference between whole blood and cell-free fluids could be demonstrated. Fairly large and equal bleeding volume deficits were observed after both types of replacement. Whether or not comparative studies show a difference in the replacement value of different fluids thus seems to depend upon the amount and type of damage done before the fluids are administered.

TABLE 3

Values are means with their standard errors. The change in liminal circulating volume is the difference between the liminal volume as measured on the final bleeding, and as estimated on the first bleeding. Positive values mean an elevation of the liminal volume on the final bleeding.

NO. DOGS	REPLACEMENT	CHANGE IN LIMINAL CIRCULATING VOLUME CC./KGM.		
		Plasma	Cells	Total
21	Gelatin sol.	13.11 ± 1.52	−4.53 ± 0.51	8.58 ± 1.86
7	Blood	2.17 ± 2.42	4.06 ± 2.22	6.23 ± 4.45

Under the present experimental conditions the efficacy of gelatin as a replacement colloid diminishes with a decrease in molecular size. In the earlier studies, maximum effectiveness was found in gelatins of intermediate grade, when a series of iso-osmolar solutions was studied. No explanation was offered for the paradoxical findings of the former studies. Both high and intermediate grades of gelatin, however, gave decreasing values for bleeding volume if they were given in highly concentrated solution (Lawson and Rehm, 1943b). It thus seemed possible that the intermediate grades produced more nearly complete reversal than the higher grades because their effective osmotic pressure happened to lie nearer the optimum, at the osmolarity chosen for the study. The study on hyperosmotic solutions in the present report represents an unsuccessful effort to extend these observations under different experimental conditions. Under the present conditions, bleeding volume is increased both by increasing the osmolarity of a particular grade of gelatin, and by increasing the average molecular size, keeping the osmolarity constant.

The present findings are in agreement with the observations of other investigators (Parkins, Koop, Riegel, Vars and Lockwood, 1944; Nicholl, Boucher and

Prince, 1945), and are in conformity with the conclusions of the National Research Council (1944). Since the optimum gelatin solution for reversing the effects of hemorrhage changes, however, with a change in the experimental conditions, it seems unwise to select on the basis of the available experimental data any single solution as optimal for clinical use.

SUMMARY

Barbitalized dogs pretreated by intravenous saline injection were almost completely exsanguinated by controlled bleeding, and injected with various replacement fluids. The fatal hemorrhage volume four hours later was used as an index to the relative effectiveness of the fluids in reversing the effects of the first hemorrhage. As measured in this manner, the order of effectiveness of the fluids studied was: whole blood > 3.9 per cent gelatin P-20 > plasma and serum > 3.45 per cent gelatin L-80 > 2.8 per cent gelatin B-20610-51 > 0.9 per cent NaCl. The gelatin solutions are approximately iso-osmolar, and have about the same colloidal osmotic pressure *in vitro* as dog plasma.

Dehydrated dogs (without saline pretreatment) were studied in similar experiments for evidence of damaging effects of gelatin P-20. The volume remaining in the circulation at death was not significantly greater than in dogs replaced with whole blood. Blood replenishment during the final hemorrhage was at least as great as in animals replaced with whole blood. The circulating blood volume maintained following the replacement was at least as great as that maintained by dogs replaced with whole blood. Replacement with higher concentrations of gelatin P-20 (6 per cent) produced larger circulating blood volumes. No evidence was obtained that the higher concentrations produced additional cardiovascular damage under these experimental conditions.

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EFFECT OF EXERCISE UPON THE ERYTHROCYTE SEDIMENTATION RATE

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With the introduction of a physical training program for convalescents into army hospitals, it is of practical importance to know more about the relationship between the erythrocyte sedimentation rate and exercise. The few references pertaining to this subject are contradictory. Cassinis (1) determined the erythrocyte sedimentation rate in eight soldiers, at rest, and after a run of from 600 to 1200 meters. He noticed a decrease in the sedimentation rate after exercise in seven cases and an increase in one. Sueda (2) found that in young and middle aged people the changes in erythrocyte sedimentation rate immediately after work are somewhat parallel to the degree of fatigue, being normal after light work and increasing with the increase of degree of fatigue.

Luciani (3) tested thirty-six children, from three to twelve years of age, twelve of whom were normal; twelve afebrile, suffering from some subacute illness; and twelve febrile, convalescing from acute illness. The exercise consisted of running 300 meters. Blood samples were taken before the run and within ten to fifteen minutes after the run. Luciani's conclusions were that this exercise produced no noticeable change in the sedimentation rate in normal children, and caused a decrease in the erythrocyte sedimentation rate in the other two groups of children, indicating that these children had a greater degree of fatigue.

Deist (4) tested 260 soldiers undergoing intensive military training. He took three samples: one during the first, the third or fourth, and the seventh weeks of training respectively. His conclusion was that there was no definite relationship between exercise and the erythrocyte sedimentation rate. Shibata et al. (5), experimenting on athletes, found that one bout of strenuous exercise caused only a slight increase in the erythrocyte sedimentation rate, whereas after a second bout the rise was more noticeable.

METHOD. The purpose of the present investigation was to determine the effect of strenuous exercise upon the erythrocyte sedimentation rate within twenty-four hours after exercise. The subjects were fifty aviation cadets, convalescing from upper respiratory infections. All patients were afebrile. The exercise used was the Harvard step-up test (6). In this test, the subject stands in front of a twenty-inch box and steps up on it with one foot and then the other, stepping down in the same order, and leading with the same foot on each step. The complete cycle is repeated thirty times a minute for as long as the subject can last, but no longer than five minutes. Work done in this exercise is equiva-

lent to going up and down 250 feet in five minutes, something like climbing to the twenty-ninth floor of a building and then coming down. All but three of the subjects in this study were able to step up and down for five minutes.

Blood samples were taken immediately before exercise, and then half an hour, five hours, and twenty-four hours after exercise. The erythrocyte sedimentation rate was determined by the Westergren method. Before exercise thirty-three patients had rates under 16 mm. and the remaining seventeen had rates ranging from 16 to 60 mm.

RESULTS. The average changes in the erythrocyte sedimentation rate at a half hour, five hours, and twenty-four hours after exercise are given in figure 1. It can be observed that there are slight elevations at the half-hour and five-hour periods after exercise and that the erythrocyte sedimentation rate returns to the pre-exercise rate after twenty-four hours. The individual variations may be comparatively large (standard deviation being 4.7 mm.) yet statistical analysis showed that for the whole group, the only significant rise in erythrocyte sedimentation rate occurred five hours after exercise (critical ratio = 3.92). These

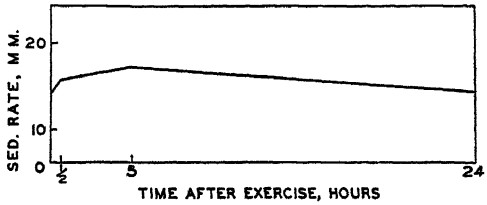

Fig. 1. Effect of strenuous exercise (Harvard step-up test for five minutes) upon the erythrocyte sedimentation rate. The curve represents the average sedimentation rates for fifty subjects. The pre-exercise rate is shown at zero (0).

conclusions hold true for patients whose pre-exercise rate was normal and for those whose rate was above normal.

The observation that the erythrocyte sedimentation rate twenty-four hours after exercise should be the same as the pre-exercise rate has been verified in another study (7). Five hundred and forty-five tests done on sixty-five subjects, who stepped up and down to the limit of their endurance, showed that only seventeen tests on eleven patients gave erythrocyte sedimentation rates above normal.

Clinical observations on the patients with high sedimentation rates failed to reveal any ill effects subsequent to the exercise and all the patients were discharged to duty within a short time after the test.

It was of interest to determine whether or not there was any relation between the erythrocyte sedimentation rate before exercise and the Harvard score. After undergoing the Harvard test, the subject receives a score which expresses the degree of his physical fitness. This score is based on the duration of exercise and the pulse rate one minute after exercise. Data on thirty-nine subjects showed that there was no relation between the erythrocyte sedimentation rate before exercise and the Harvard score ($r = -0.15$).

SUMMARY AND CONCLUSIONS

1. Fifty aviation cadets, convalescing from upper respiratory infection, were given five minutes of strenuous exercise (the Harvard step-up test).

2. Blood samples were taken immediately before exercise, and half an hour, five hours, and twenty-four hours after exercise. The erythrocyte sedimentation rate was determined by the Westergren method.

3. Five minutes of strenuous exercise caused a variable effect upon the individual sedimentation rate. However, in the group tested there was a statistically significant increase in erythrocyte sedimentation rates five hours after exercise but no significant change either half an hour or twenty-four hours after exercise. This indicates that the change is not lasting.

4. Post-exercise observations in no case showed any clinical manifestations of ill effects.

5. Data on thirty-nine subjects showed that there was no relation between the erythrocyte sedimentation rate before exercise and the score obtained on the Harvard step-up test ($r = -0.15$).

6. The contradictions regarding the effect of exercise upon the erythrocyte sedimentation rate found in the literature may be explained by the difference in the intensity of exercises and in the time of taking the blood samples.

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THE EFFECT OF BED REST ON THE BLOOD VOLUME OF NORMAL YOUNG MEN[1]

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It is generally agreed that rest in bed produces a decline in physical "fitness" associated with a deterioration of cardiovascular function. In any analysis of the causes of changing cardiovascular function it is necessary to consider the volume of the circulating blood. It is the purpose of this paper to present data concerning the effect of bed rest on blood volume.

Materials and conditions. The subjects were volunteers from Civilian Public Service. These men were from 20 to 32 years of age and were free from present and significant past disease.

The general plan of the experiment involved a preliminary training and standardization period, a 3-week period of bed rest and a 6-week period of reconditioning. Under continuous supervision, the men followed a definite routine while in bed. They were allowed to get up once a day for bowel movements, it being considered that this involved less effort than the use of the bedpan. Complete inactivity (bed flat, dim light) was enforced for 4 daytime hours, divided into three periods. Routine "bed-fast" nursing care was provided by a registered nurse. Lights were turned out at 10 p.m.

Pre- and post-bed rest conditioning was carried out on a motor-driven treadmill. A definite schedule of walking at 3.5 miles per hour and a 10 per cent grade was maintained by the subjects 5 days a week during "active" periods. This rate of work demands an oxygen consumption of approximately 8 times the basal metabolic rate. At regular intervals the men performed bouts of "anaerobic" work, i.e., running at 7 miles per hour and 10 per cent grade for 3 minutes and running at the same rate on a 15 per cent grade for 90 seconds. All work was carried out in an air-conditioned suite maintained at 78°F. and 50 per cent relative humidity. Standard clothes and shoes were worn for all test sessions. The total caloric expenditure per day for the week before going to bed was 4500 calories. On getting out of bed the total daily caloric expenditure was increased from 2500 calories by weekly steps of 500 calories up to the original level. The men were in continuous residence in the laboratory dormitory or metabolism ward as the occasion demanded. The subjects were fed a diet of known composition which was "adequate" in protein, calories and vitamins. Body weight remained substantially constant at all times before, during and after bed rest.

[1] The work described in this paper was done in part under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Minnesota. Important financial assistance was also provided by the Nutrition Foundation, Inc., New York and the National Dairy Council, Chicago.

Methods. A procedure modified from that of Gibson and Evelyn (1) was followed in determining blood volume. The concentration of the dye was determined on 6 ml. samples of plasma in the standard Evelyn photoelectric colorimeter. After a dye-free blood sample had been drawn, 15 mgm. of T-1824 (Evans' Blue) dye dissolved in 10 ml. of saline were injected into an antecubital vein in 40 seconds. The concentration of dye in the plasma was determined in blood samples drawn at 20, 30 and 40 minutes after injection. The "K" value for the dye was determined in each dye-free sample to reduce the possibility of error due to changes in the optical properties of the plasma (2, 3) which may occur in different states. All blood was drawn in oiled syringes and was centrifuged in waxed tubes using heparin as an anticoagulant. Since only relative changes were under consideration, blood volumes were calculated directly from the hematocrit without making a correction for the error in the hematocrit determination (4) or for the greater proportion of plasma in the capillary blood as compared to venous blood (5).

All determinations of blood volume were carried out in the morning under basal conditions. Subjects were not allowed out of bed for one half hour before the injection of dye to avoid plasma volume changes due to posture or activity. Except in those cases noted, the values reported here are in each case the average of 2 determinations on separate days. The standard deviation of any single determination from the average presented in the tables was found to be $\pm$ 84 ml. of plasma (statistical analysis of 16 pairs of duplicates).

Pulse rates were counted 3 times with a stethoscope during the last five minutes of a routine one hour period of "aerobic" work (3.5 m.p.h. at 10 per cent grade).

Results. The data on the effect of bed rest on the blood volume and its component parts are presented in table 1 together with the work pulse rates. There was an average decrease of 15.5 per cent in plasma volume while the average total volume of circulating red cells remained almost unchanged. There was an average decrease of 9.3 per cent in the total circulating blood. The declines in plasma volume were consistent in all cases. On the other hand in one case a large rise in the hematocrit indicated an increase in red cell volume large enough to offset the plasma loss and to result in an apparent increase in the total circulating blood volume. In 4 men the plasma and blood volumes were studied in the course of reconditioning after bed rest. The data are presented in table 2 along with the work pulse rates. It will be noted that a loss of red cells occurred in the first week but this was more than offset by a large rise in plasma volume so that there was an average net gain of 235 ml. in the total circulating blood volume at this time. During the subsequent period of reconditioning the average net gain of 420 ml. of whole blood was almost entirely attributable to an increase in the red cell volume. The work pulse rates presented in tables 1 and 2 are discussed below.

Subject E. S. volunteered to submit to surgical repair of his right inguinal hernia. This procedure was carried out at the end of the 72nd day of training after the completion of three weeks in bed. He was kept in bed under the same restrictions for the same length of time as in the preceding experiment. His

plasma volume decreased 628 ml. after surgery as compared to 769 ml. for simple bed rest and his total blood volume decreased 360 ml. after surgery as compared to 733 ml. after simple bed rest.

DISCUSSION. The limitations of the method employed for the measurement of blood volume should be recognized. However it can be pointed out that there is no evidence in the data that the observed changes are due to an increased rate of loss of plasma proteins to the lymph (6, 7). The slope of the disappearance curve of the dye was 0.015 before and 0.014 after bed rest. It follows then that the calculation of the plasma volume from the dye concentration in the

TABLE 1

The plasma volume, the red cell volume, total blood volume, the hematocrit and the work pulse rate (walking at 3.5 m/h and 10% grade) before and after a three week period of bed rest

EXP.	SUBJECT	PERIOD	PLASMA VOLUME IN ML.	HEMATOCRIT PER CENT	RED CELL VOLUME IN ML.	BLOOD VOLUME IN ML.	BLOOD VOLUME DIFFERENCE IN ML.	PULSE BEATS/MIN.	WORK PULSE DIFF. BEATS/MIN.
1	R. M.	Before bed rest	2705	46.8	2378	5083		126	
		After bed rest	2585	52.0	2803	5390	+307	171	+45
2	D. M.	Before bed rest	3582	46.5	3122	6704		123	
		After bed rest	2850	51.2	2989	5839	−865	181	+58
3	A. W.	Before bed rest	3598	48.0	3325	6923		134	
		After bed rest	3077	47.0	2729	5806	−1117	167	+33
4	E. S.	Before bed rest	4020	42.7	2994	7014		121	
		After bed rest	3251	48.2	3030	6281	−733	162	+41
5	L. B.	Before bed rest	2974	44.5	2400	5374		118	
		After bed rest	2679	48.3	2515	5194	−180	163	+45
6	R. M.	Before bed rest	3127	45.6	2616	5743		117	
		After bed rest	2449	50.0	2448	4897	−846	168	+51
Average.......		Before bed rest	3334	45.7	2806	6140			
		After bed rest	2816	49.5	2752	5568			
Average......			−518	+3.8	−54	−572			

20-minute sample would lead to the same conclusion as the use of the extrapolated dye concentration. Plasma volumes calculated in this way were 4.2 per cent larger than those in tables 1 and 2 and the average decrease in bed rest was only 14 ml. smaller than the decrease reported above where the extrapolated values of dye concentration were used. The product moment correlation (8) between the extrapolated and 20-minute dye concentrations was 0.966.

It is not our purpose here to discuss the whole course of the large deterioration in cardiovascular function which occurred as the result of bed rest. This will be done elsewhere. The work pulse rates are presented merely as an illustration.

Attempts were made to assess the rôle that the loss of blood volume played in the observed alterations in cardiovascular function. Inspection of the aerobic work pulse rate changes shows no close correlation with the blood volume loss or gain. For example, subject R. M. in experiment 1 showed as the result of 3 weeks in bed a slight increase in blood volume and a work pulse rate increase that was greater than that of E. S. who apparently lost 733 ml. of blood. In experiment 7 when R. M. repeated the stay in bed, he had a marked loss of blood and his work pulse increased only 6 beats per minute. Limitations of space

TABLE 2

The plasma volume, blood volume, and work pulse rate (walking at 3.5 m/h and 10% grade) in 4 men at different periods during reconditioning

"Bed rest" refers to the end of bed rest, "one week" to the end of the 1st week of activity and "final" refers to a reconditioning period of 46 days for L. B. and G. W. and 28 days for R. M. and D. M. Blood and plasma volume and their respective changes are expressed in ml.

EXP. NO.	SUBJECT	PERIOD	PLASMA VOLUME	BLOOD VOLUME	BLOOD VOLUME CHANGE		WORK PULSE IN BEATS/MIN.	WORK PULSE DIFFERENCE	
					Bed rest 1st week	1st week to final		End of bed rest to 1st week	1st week to/incl.
5	L. B.	Bed rest	2679	5194			162	11	
		One week*	3263	5457	+263		151		
		Final	3379	6352		+895	125		26
6	G. W.	Bed rest	2458	4642			167		
		One week*	2990	5042	+400		151	16	
		Final	2907	5285		+243	127		24
7	R. M.	Bed rest	2449	4897			171		
		One week*	2710	4570	−327		145	26	
		Final	3074	5480		+910	129		16
8	D. M.	Bed rest	2916	5464			174		
		One week*	3726	6068	+604		139	35	
		Final	3230	5697		−371	124		15

* These values represent a single determination.

prevent the presentation of data which show that a similar lack of correlation exists between blood volume loss or gain in ml. per kilo and the following items:[2] the increase in lactate and recovery pulse after a fixed task of "anaerobic" work; the change in heart size; the increase in resting pulse rate; and the postural adjustment score or the several items which go into it. The loss of oxygen transported per kilo of body weight during a 90 second "anaerobic" run did show a correlation of 0.75 (with blood volume loss) as the result of bed rest. It is felt that more data are needed to establish this relationship.

[2] The detailed results will be presented elsewhere.

The apparent lack of correlation between blood volume loss and certain cardiovascular functions is not surprising in view of the uncertainty in blood volume measurements. The changes are not large and represent the difference between 2 measurements whose individual uncertainties are, at the very best, ± 2 per cent. Also the number of individuals examined is small. Finally the possibility of artifact in the dye method which might invalidate inter-individual comparison cannot be completely ignored (7, 9, 10). It is evident that the deterioration in cardiovascular function induced by bed rest was accompanied by a loss of blood volume and that on completion of the reconditioning program the blood volume had increased to approximately pre-bed rest levels. Karpovitch (11) has studied the effect of withdrawal of 500 ml. of blood on endurance in trained athletes. The performance on the bicycle ergometer of the 5 men studied deteriorated and the original level was not regained for about three weeks. These results are open to the criticism that it was not possible to control the effects of suggestion.

Blood depots which are generally believed not to be large in man (12–15) apparently played no part in the decrease in circulating blood volume produced by bed rest since there was no significant change in the circulating red blood cells. The apparent loss of red cells during the 1st week of activity is similar to the loss of red cells in untrained dogs during a period of training (16). This has been shown to be due to actual destruction of red cells (17, 18, 19, 20) and presumably there was a loss of red cells in our subjects due to the same cause.

The loss of blood volume in milliliters per kilogram of body weight averaged 9.3 ml. (range +4.6 to −14.0 ml.). The changes in blood volume with the state of physical condition helps to explain the inter-individual variations reported by Gibson and Evans (22).

It is interesting that the decrease of plasma volume was accompanied by a negative nitrogen balance during bed rest and the increased plasma volume in the early part of the period of reconditioning by a positive nitrogen balance (21).

It should be recognized that the men studied here were in good physical condition. It is considered that extremely sedentary individuals might exhibit quantitatively different responses.

The validity of claims of increases in plasma or blood volume in disease states (23) must rest on the demonstration that plasma volumes of patients during the disease state are higher than plasma volumes of the same convalescent patients who have reached a state of physical condition comparable to the predisease state.

SUMMARY

1. The effect of 3 weeks of complete bed rest on the blood volume and its component parts has been studied in 6 experiments on 5 normal young men. In 4 men studies were carried out during the course of reconditioning. In addition one of these men was studied before and after the surgical repair of an inguinal hernia.

2. An average loss in blood volume of 572 ml. or 9.3 per cent occurred during

the period of bed rest. This was almost entirely accounted for by a contraction of the plasma volume of 518 ml., or 15.5 per cent.

3. The first week of reconditioning resulted in an increase in plasma volume to pre-bed rest levels but was accompanied by an apparent loss of red cells so that the average increase of blood volume was only 235 ml. The subsequent apparent increase in blood volume to the original level was due entirely to an increase in red blood cells.

4. The blood volume change after the surgical repair of an inguinal hernia and 3 weeks' bed rest in one man did not differ significantly from the changes observed in the same man after simple bed rest alone.

5. Correlations between blood volume changes and various indices of deterioration of cardiovascular function are discussed.

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THE ABILITY OF THE LIVER TO CHANGE BLOOD GLUCOSE AND LACTATE CONCENTRATIONS FOLLOWING SEVERE HEMORRHAGE[1,2]

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In a previous paper (1) the increase in the blood concentrations of lactate, glucose and pyruvate which occurs after a severe hemorrhage was described. These changes were interpreted as showing a progressive increase, followed by a decrease, in the peripheral utilization of glucose. The lactate concentration increased immediately after the hemorrhage and a marked rise occurred terminally. These changes in blood glucose and lactate concentrations reflect a disturbance in the glycolytic cycle. According to current concepts (2) the liver, during this cycle, releases glucose to the blood stream. The blood glucose is removed by the muscles and metabolized to lactate which is returned to the liver where it is resynthesized to glycogen. The purpose of the present experiments was to determine how efficiently the liver functions with respect to the glycolytic cycle during the hypoxic period following a severe hemorrhage.

METHOD. The experiments were done on dogs of 7 to 15 kilos, fasted for 18 hours previous to the experiment (in the postabsorptive state). A week or more before the experiment a cannula was placed on the portal vein, according to the method of Tsai (3). Hepatic venous samples were obtained by introducing a catheter directly into the liver through the left jugular vein (local anesthesia). No difficulty was encountered in introducing the catheter, which was removed after each blood sample was taken. The distance that the catheter must travel from the external jugular vein to the liver was estimated quite precisely before insertion and the "feel" of the catheter when it was securely in a liver lobule was unmistakable. As a further check, the position of the catheter in each animal was determined either by fluoroscopy or at autopsy. During the control period the insertion of the catheter did not appear to interfere with the circulation of the animal. However, when the blood pressure was at a low level after bleeding the insertion of the cannula often caused a rapid fall in the blood pressure followed by a return to the previous level when the catheter was removed. Within a period of 2 minutes samples were drawn from the femoral artery and vein and from the portal and hepatic veins. This was done before and at hourly intervals after a 70 per cent hemorrhage (bled out according to Walcott's method and 30

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[2] Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, in the Faculty of Pure Science, Columbia University.

per cent of the bleeding volume returned) (4). Analyses of lactate and glucose were carried out as described in a previous paper (1). The femoral arterial-venous (A-V) difference is assumed to represent utilization of glucose or production of lactate by the muscles of the hind limb; the arterial-portal (A-P) difference, utilization of glucose or production of lactate by the viscera. The arterial-hepatic venous (A-H) and the portal-hepatic venous (P-H) differences indicate the changes produced by the liver as the blood passes through this organ. A minus sign has been placed before the A-V, A-P, A-H, or P-H difference when the tissues remove glucose or lactate, and a plus sign when the tissues add glucose or lactate.

Results and discussion. Control determinations on dogs in the postabsorptive state (table 1) indicate that glucose was generally removed by the muscles and viscera, and released by the liver, as has been demonstrated by several investigators (5). After a 70 per cent hemorrhage the arterial glucose level rose to a maximum and then decreased progressively until death. The P-H differences increased an average maximum of 10 times the control value, the A-H differences 15 times the control value, after which both differences decreased progressively until death occurred. Thus as the blood passes through the liver there is a greater increase in the glucose concentration following hemorrhage than in the controls. The arterial level of glucose can be decreasing at the same time that the hepatic output is increasing, as shown by dog 1 between the first and second hour after hemorrhage. This can be explained in terms of an increase in the peripheral utilization of glucose (1), the level of arterial glucose being determined both by the relative amount of glucose produced by the liver and by the magnitude of the peripheral utilization. Glucose utilization by the leg muscles (A-V) and the viscera (A-P) generally rose to a maximum and then fell progressively until death occurred.

It is impossible to determine the total amount of glucose supplied to the blood by the liver per unit time without blood flow measurements. Blalock (6) measured the hepatic blood flow following moderate hemorrhage in unanesthetized dogs and found a decrease of about 50 per cent. In the present experiments the blood flow to the liver probably fell to somewhat below 50 per cent of the control value. In addition, the effect of bleeding upon the relative amount of blood supplied to the liver by the hepatic artery and by the portal vein are undetermined and these values are known to vary widely even in control dogs (7). Therefore the formula 3P + A/4-H (3 times the portal blood concentration plus the arterial blood concentration divided by 4 and minus the hepatic venous blood concentration) which is based on the usual assumption that the hepatic artery contributes $\frac{1}{4}$ and the portal vein $\frac{3}{4}$ of the blood entering the liver, cannot be used to calculate the overall changes in the glucose or lactate concentration as the blood passes through the liver, but either the P-H or the A-H differences are indices of these changes in concentration.

In the control dogs in the postabsorbtive state lactate was generally produced by the muscles (A-V) and the viscera (A-P) and removed by the liver (A-H, P-H, table 2), and has been demonstrated by other investigators (8). If the

TABLE 1

Arterial glucose levels in milligrams per cent compared with the changes in glucose concentration as the blood passes through muscle, liver and viscera

DOG	CONTROL		HOURS FOLLOWING HEMORRHAGE			
			1 hour	2 hours	3 hours	4 hours
1	Art.	66.0	111.1	83.5	56.0	14.0*
	A-V	0.0	−23.0	−21.1	−9.0	
	P-H	+15.5	+37.0	+49.0	+34.6	
	A-H	+13.5	+12.0	+30.5	+14.0	
	A-P	−2.0	−25.0	−18.5	−20.6	
2†	Art.	66.7	312.0	153.0	Dead 2 hours	
	A-V	−3.7	−27.0	−10.0		
	P-H	+6.4	+52.0			
	A-H	−5.3	+37.0	+15.0		
	A-P	−11.7	−89.0			
3	Art.	76.2	263.0	203.0	Dead 2 hours	
	A-V	−4.2	−46.0	−3.0		
	P-H	0.0	+56.0	+24.0		
	A-H	0.0	+44.0	+21.0		
	A-P	0.0	−12.0	−3.0		
4	Art.	56.0	220.0	218.0	130.0‡	120.0§
	A-V	−2.0	−35.0	−21.0	−12.0	−1.0
	P-H	+6.0	+26.0	+31.0	+48.0	+80.0
	A-H	+6.0	+3.0	+13.0	+4.0	+48.0
	A-P	0 0	−23.0	−18.0	−44.0	−32.0
5¶	Art.	76.0	178.0	335.0	168.0	128.0
	A-V	−10.0	−19.0	−22.0	−19.0	−3.0
	P-H	+6.0	+46.0	+86.0	+28.0	+38.0
	A-H	+1.0	+30.0	+66.0	+8.0	+18.0
	A-P	−5.0	−16.0	−20.0	−20.0	−20.0
6‖	Art.	90.9	263.0	258.0	438.0	Dead 3 hours
	A-V	−1.8	−22.0	−14.0	−25.0	
	P-H	+11.0	+27.0	+25.0	+205.0	
	A-H	+3.2	+17.0	−10.0	+177.0	
	A-P	−7.8	−10.0	−35.0	−28.0	

* Death occurred immediately following the sample.

† Seventy-five per cent hemorrhage.

‡ Four hour samples.

§ Five hour samples, survival period 5¾ hours.

¶ Survival period of five hours.

‖ Hemorrhage interrupted before bleeding-out volume reached. Forty cubic centimeters extra blood removed at 2½ hours.

P-H differences are used for calculation the liver in the postabsorptive state removed between 0 and 48 per cent of the portal blood lactate. In a statistical

TABLE 2

Arterial lactate level in milligrams per cent compared with the changes in lactate concentration as the blood passes through the muscle, liver and viscera

DOG	CONTROL		HOURS FOLLOWING HEMORRHAGE		
			1 hour	2 hours	3 hours
7	Art.	19.1	91.4	98.6	Dead 2½ hours
	A-V	+1.8	+13.6	+42.0	
	P-H	0.0	+3.8	−8.0	
	A-H	0.0	+3.2	+24.4	
	A-P	−0.1	−0.6	+32.4	
8	Art.	4.1	55.5	95.5	128.1*
	A-V	+3.3	+30.8	+14.5	
	P-H	−3.2	−20.7	−11.4	−8.5
	A-H	−1.4	−4.8	+1.1	+5.9
	A-P	+1.8	+15.9	+12.5	+14.4
9	Art.	10.0	102.0	Dead 1½ hours	
	A-V	+2.1	+31.0		
	P-H	−1.1	−18.0		
	A-H	−0.2	+25.0		
	A-P	+0.9	+43.0		
10	Art.	11.5	120.3†	155.5‡	Dead 1½ hours
	A-V	+1.2	+6.5		
	P-H	−2.5			
	A-H	−2.0	−7.2	−1.7	
	A-P	+0.5			
11	Art.		151.1	Dead 1¾ hours	
	A-V	−3.3			
	P-H		−2.0		
	A-H		−1.0		
	A-P				
12	Art.	10.6	52.4	70.2	86.0§
	A-V	+2.0	+24.8	+19.8	+39.0
	P-H	−6.5	−18.4	−20.9	
	A-H	−1.1	−1.3	−16.6	
	A-P	+5.4	+17.1	+4.3	+17.0
13	Art.	8.5	131.0	160.0¶	
	A-V	+3.0	+14.5		
	P-H	−4.2	+5.0	+3.0	
	A-H	+1.7	+7.0	+27.0	
	A-P	+5.9	+2.0	+24.0	

* Dead 3½ hours.
† One-half hour sample.
‡ One hour sample.
§ Two and one-half hour sample, dead 2½ hours.
¶ One and one-half hour sample, dead 1½ hours.
Dogs 10, 11, and 12 were subjected to a 75 per cent hemorrhage,

analysis of a much larger series of unanesthetized dogs in the postabsorptive state, Cherry and Crandall (8) using the formula 3P + A/4-H found no significant difference in the average lactate concentration of blood passing through the liver. Nevertheless, in certain dogs it is clear that in the postabsorptive state the liver can remove lactate although this is not revealed in a statistical analysis of the change in lactate concentration as the blood passes through the liver. For example, in dog 12 the control P-H difference was −6.5 mgm. per cent and the overall hepatic difference in lactate concentration using the formula 3P + A/4-H was −5.2 mgm. per cent.

In the first hour following a 70 per cent hemorrhage the amount of lactate removed from the portal blood by the liver as measured by the P-H differences may or may not increase above the control value (table 2). Even when the P-H difference increases above the control value, it does not necessarily mean that the fraction of lactate removed from the portal blood (0 to 29 per cent) is greater than that removed during the control period (0 to 48 per cent), since the concentration of lactate in the blood has risen following hemorrhage. The arterial lactate concentration rose throughout the entire experiment. After the first hour both the A-H and the P-H differences indicated a progressive decrease in the amount of lactate removed by the liver. The initial increase in the peripheral and visceral release of lactate is succeeded by a period during which the production of this substance may either continue to increase, decrease, or even remain unchanged.

SUMMARY

1. After a 70 per cent hemorrhage (bled out and 30 per cent of the bleeding volume returned) there is an increase in the amount of glucose released by the liver increasing the arterial glucose level in spite of the rise in peripheral glucose utilization.

2. After reaching a maximum value, the portal-hepatic venous and the arterial-hepatic venous differences decrease until death occurs.

3. The ability of the liver to remove lactate varies. During the first hour after bleeding (70 per cent hemorrhage) the actual portal-hepatic venous difference may rise as high as −20.7 mgm. per cent. However, when this increase in the amount of lactate removed by the liver occurs, it does not mean that the fraction of the lactate removed (0 to 29 per cent) is necessarily greater than that removed during the control period (0 to 48 per cent). As the hypoxia continues the ability of the liver to remove the lactate presented to it fails progressively and the arterial level of lactate rises accordingly.

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THE NATURE OF THE RENAL TUBULAR MECHANISM FOR ACIDIFYING THE URINE

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In the human the average dietary intake of potential acid exceeds the intake of available base by some 30 to 80 m.eq. per day. This excess acid is neutralized by base derived from the bicarbonate of the body fluids, and if it were excreted in combination with this base, the alkali reserve of the body would be rapidly depleted. Two types of renal compensatory mechanisms provide for the excretion of acid without the loss of equivalent amounts of fixed base. 1. The renal tubules convert the slightly alkaline glomerular filtrate into acid urine, and because acid is excreted in part in the free form, a saving of base is effected. This mechanism normally accounts for the excretion of 10 to 30 m.eq. of acid per day. 2. The renal tubules synthesize ammonia and convert the sodium salts present in the glomerular filtrate in part to ammonium salts, in which form they are excreted. An equivalent amount of fixed base is retained in the body. This mechanism normally accounts for the excretion of 20 to 50 m.eq. of acid per day.

The ability of the kidney to excrete an acid urine and thus to conserve base depends upon the quantity of buffer available to the kidney. Normally this buffer consists largely of phosphate. In plasma at pH 7.4 phosphate exists as a mixture of Na_2HPO_4 and NaH_2PO_4 in a ratio of 4 parts of the dibasic salt to one part of the monobasic salt. In the most acid urine which can be excreted, namely, a urine of pH 4.8, practically all of the phosphate exists in the monobasic form. Thus per mol of phosphate excreted at this pH, 0.8 equivalent of base is conserved. At a pH of 4.8 only insignificant amounts of free hydrochloric and sulfuric acids can exist. Hence base conservation in excretion of these acid anions must be effected wholly by the conversion of sodium salts to ammonium salts. These general principles are well reviewed by Peters and Van Slyke (12) and by Gamble (7) and need no further comment here.

The process by which the renal tubules acidify the urine is the subject of the present investigation. The general reaction,

$$\underset{}{Na_2HPO_4} + H_2CO_3 \longrightarrow \underset{\text{(excreted)}}{NaH_2PO_4} + \underset{\text{(reabsorbed)}}{NaHCO_3}$$

is ordinarily considered in studies of acid base metabolism to be an adequate treatment of the renal contribution to the problem. Actually three theories as to the nature of the renal processes involved have been postulated, all based upon this fundamental reaction. Figure 1 illustrates diagrammatically the concepts involved in these theories.

1. *The phosphate reabsorption theory.* An ultrafiltrate of plasma containing Na_2HPO_4 and NaH_2PO_4 in a ratio of 4:1 is formed at the glomeruli. According to the phosphate reabsorption theory, the NaH_2PO_4 is excreted, whereas the Na_2HPO_4 is reabsorbed by the renal tubules and returned to the postglomerular blood. The dibasic phosphate which is reabsorbed reacts in part with the free carbonic acid of blood to restore the normal ratio of dibasic and monobasic phosphates (Peters and Van Slyke, 12).

2. *The carbonic acid filtration theory.* In addition to phosphate, the glomerular filtrate contains $NaHCO_3$ and H_2CO_3 in a ratio of 20:1. According to the carbonic acid filtration theory, the renal tubules are completely impermeable to H_2CO_3, whereas they actively reabsorb $NaHCO_3$. Thus the reaction between

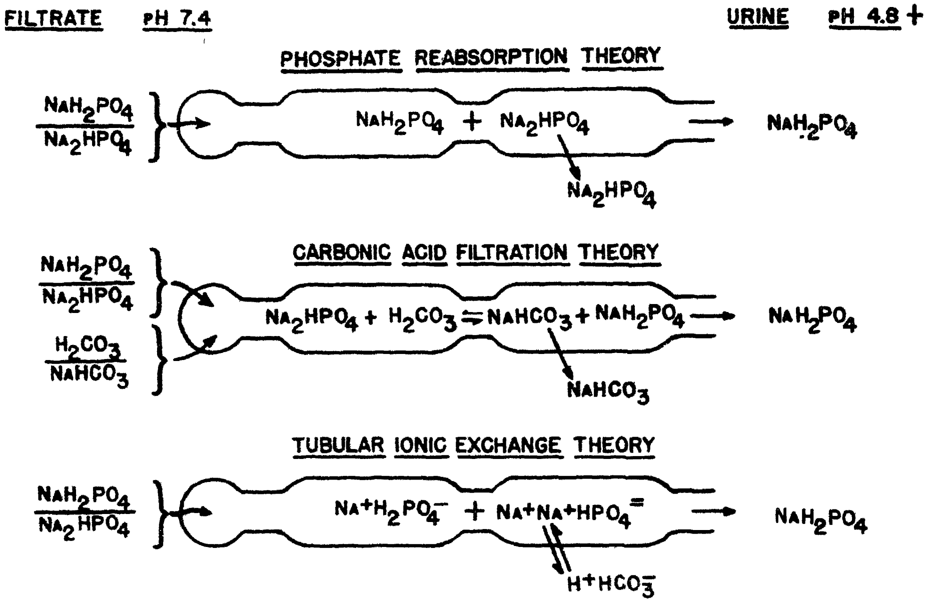

Fig. 1. Theories of the nature of the renal mechanism for acidifying the urine. In each diagram the kidney is represented as a single nephron composed of a glomerulus on the left, followed by a renal tubule consisting of proximal and distal segments.

dibasic phosphate and carbonic acid occurring in the lumen of the renal tubules is forced to the right by the reabsorption of $NaHCO_3$, and the monobasic phosphate which is formed is excreted in the urine (Sendroy, Seelig, and Van Slyke, 14).

3. *The tubular ionic exchange theory.* According to this theory, the significant constituents of the glomerular filtrate are monobasic and dibasic phosphate. Bicarbonate and carbonic acid are both reabsorbed in large part and accordingly need not be considered. In the passage of the filtrate down the tubules H^+ ions derived from the tubular cells are exchanged for Na^+ ions in the tubular lumen, converting the dibasic phosphate to the monobasic form. The source of the H^+ ions is H_2CO_3 produced within the tubular cells or derived by them from the carbon dioxide of the postglomerular blood (Smith, 15).

Any one of these three theories explains adequately the titratable acidity and the pH of normal urine. However, each theoretical mechanism has inherent within it a specific limitation of its capacity to effect the excretion of acid. According to the phosphate reabsorption theory and the carbonic acid filtration theory all of the acid which appears in the urine was initially filtered through the glomeruli. In the former theory NaH_2PO_4 constituted the filtered acid; in the latter theory H_2CO_3 constituted the filtered acid. According to the ionic exchange theory, acid is added to the glomerular filtrate by the renal tubules. Thus the first two theoretical mechanisms are limited by the quantity of acid present in the original filtrate, whereas the third is limited by the quantity of acid which the tubules can add to this filtrate. It occurred to us that one might utilize these specific limitations of capacity as a means of identifying the true nature of the mechanism for acidifying the urine. Accordingly we have stimulated the renal acidifying mechanism in dogs by the repeated feeding of hydrochloric acid, and have provided the animals with a large excess of buffer in continuous intravenous infusions. We have found that the titratable acidity of the urine under such experimental conditions is so great that it can be explained only by a mechanism which involves the tubular transport of acid. Biologically, the most reasonable mechanism of this type is the one which effects an exchange of H^+ ions for Na^+ ions across the renal tubular epithelium.

METHODS. In all, 18 experiments were performed on 4 trained female mongrel dogs weighing between 16 and 20 kgm. The animals were rendered acidotic by the daily administration of 500 cc. of 1 per cent hydrochloric acid by stomach tube for at least 4 days preceding the day of the experiment. This amount of acid was likewise given at the beginning of each experiment. Unfortunately the loss of variable amounts of acid by vomiting caused considerable variations in the degree of acidosis attained. The dogs were fasted for 18 to 24 hours prior to the experiment. During an experiment the animals were loosely restrained on a comfortable animal board. Urine was collected by catheter and the bladder was washed out with 10 cc. of distilled water at the end of each urine collection period. Bloods were drawn from the jugular vein or from an indwelling needle in the femoral artery at the mid-point of each urine collection period.

Creatinine and sodium phosphate (pH 7.4) were administered in continuous intravenous infusions into the saphenous vein at rates of 5 or 10 cc. per minute. The plasma concentration of creatinine was maintained relatively constant during the course of an experiment at levels of 20 mgm. per cent or more to ensure accuracy of determination in both plasma and urine. The clearance of creatinine has been used as a measure of the rate of formation of glomerular filtrate. The quantities of phosphate or carbonic acid filtered through the glomeruli in a minute's time were calculated as the product of the glomerular filtration rate in cubic centimeters per minute and the plasma concentration of phosphate and carbonic acid in millimols per cubic centimeter. The quantity of phosphate reabsorbed was calculated as the difference between the quantity filtered and the quantity excreted (13).

In experiments 1 to 6, arterial bloods were drawn in oiled syringes and delivered under oil into centrifuge tubes. The tubes were completely filled with

blood and sealed with tight fitting rubber stoppers. Minimal quantities of neutral potassium oxalate and fluoride were used to prevent clotting and glycolysis. After centrifugation the carbon dioxide content of the plasma was determined by the method of Van Slyke and Sendroy (19). Analyses were made within a few hours and plasmas were iced until analyzed. Arterial pH was determined electrometrically on samples of whole blood drawn directly into a hypodermic type glass electrode without exposure to air. The electrode when filled was cooled rapidly to room temperature by immersion in a water bath. The blood pH was immediately determined at room temperature and corrected to 38°C. by subtracting 0.014 pH unit per degree difference in temperature (11). In other experiments (7 to 10) the carbon dioxide combining power of venous blood was determined by the method of Van Slyke and Neill (18). The pH of urine was determined electrometrically, and the titratable acidity of the urine was determined by titrating it to the observed pH of the arterial plasma. In experiments in which venous bloods were drawn and pH measurements on arterial blood were not available, the urines were titrated arbitrarily to pH 7.35. Two errors of small magnitude enter into these measurements on urine: pH measurements were made at room temperature and were not corrected to body temperature; no precautions were taken to prevent loss of carbon dioxide from the urine. Since the urines for the most part were acid and strongly buffered, and since the titrations were begun and ended at room temperature, the errors introduced were small and have been disregarded. The preparation of plasma filtrates and the analysis of creatinine and phosphate have been described in a previous communication (13). Sulfanilamide was analyzed by a modification of the method of Bratton and Marshall (2) on trichloracetic acid filtrates of both plasma and urine.

RESULTS. *Proof of the tubular transport of acid.* Experiments presented in tables 1 and 2 demonstrate that if conditions are properly chosen, the quantity of titratable acid excreted in the urine may far exceed the quantity of acid filtered through the glomeruli. These experiments therefore prove the existence of some tubular mechanism for the transportation of acid from the postglomerular blood into the tubular lumen. In 4 experiments shown in table 1, from 0.198 to 0.380 m.eq. of titratable acid were excreted per minute, roughly equivalent to 3000 to 6000 cc. of 0.1 N acid per day. The acidosis and the high rate of excretion of phosphate are the significant factors which account for the high titratable acidity. The acidosis in our experimental animals was moderate, as is indicated by arterial CO_2 contents of 14 to 18 mM. per l. The normal value for the dog is 23 to 26 mM. per l. The arterial blood pH was somewhat reduced, perhaps as a result of the continued absorption of acid throughout the course of the experiment. However an important factor determining the high rate of acid excretion is the high rate of phosphate excretion. To attain excretion rates of approximately 0.5 mM. per min. the plasma concentration was elevated to some 10 times the normal value by the continuous infusion of phosphate.

In the latter half of table 1 is presented a critical analysis of the data in terms of the titratable acidity of the urine. The values for observed titratable acidity

TABLE 1

Experiments on acidotic dogs designed to test critically the several theories of the nature of the renal mechanism for acidifying the urine

The major urinary buffer in these experiments was phosphate

TOTAL CONCURRENT TIME	ARTERIAL PLASMA CONCENTRATION				GLOMERULAR FILTRATION RATE	URINE		PHOSPHATE			CREATININE EXCRETED	TITRATABLE ACIDITY OF URINE								
												Observed	Calculated from							
	Creatinine	Phosphate	Total CO_2	pH		Flow	pH	Filtered	Excreted	Reabsorbed			Phosphate excreted	Creatinine excreted	Total buffer excreted		Phosphate reabsorption theory		Carbonic acid filtration theory	
min.	*mgm. %*	*mM./l*	*mM./l*		*cc./min.*	*cc./min.*		*mM./min.*	*mM./min.*	*mM./min.*	*mM./min.*	*mEq./min.*	*mEq./min.*	*mEq./min.*	*mEq./min.*	*% of observed*	*mEq./min.*	*% of observed*	*mEq./min.*	*% of observed*
Experiment 1; dog 5																				
105–120	26.4	8.37	18.7	7.34	73.1	7.60	6.06	0.612	0.489	0.123	0.171	0.310	0.300	0.012	0.312	100.6	0.028	9.0	0.075	24.2
120–135	24.8	8.41	18.7	7.34	76.4	7.93	6.02	0.642	0.514	0.128	0.168	0.340	0.326	0.013	0.339	99.7	0.029	8.5	0.078	22.9
135–150	23.9	8.53	18.6	7.37	76.9	7.53	6.26	0.656	0.525	0.131	0.163	0.298	0.293	0.007	0.300	100.6	0.028	9.4	0.073	24.5
150–165	25.0	9.00	18.5	7.39	75.1	8.73	6.32	0.676	0.538	0.138	0.166	0.297	0.294	0.006	0.300	101.0	0.028	9.4	0.068	22.9
Experiment 2; dog 6																				
115–130	29.1	7.22	15.0	7.32	72.2	7.53	6.14	0.521	0.404	0.113	0.186	0.240	0.240	0.011	0.251	104.5	0.026	10.8	0.061	25.4
130–145	28.2	7.32	14.7	7.32	67.8	7.53	6.28	0.496	0.396	0.100	0.169	0.215	0.213	0.007	0.220	102.3	0.023	10.7	0.057	26.5
145–160	29.4	7.93	14.4	7.33	61.2	8.00	6.35	0.485	0.384	0.101	0.159	0.198	0.195	0.006	0.201	101.5	0.023	11.6	0.049	24.7
160–175	31.8	8.69	14.0	7.34	56.0	8.40	6.38	0.486	0.389	0.097	0.157	0.198	0.195	0.005	0.200	101.0	0.022	11.1	0.042	21.2
Experiment 3; dog 2																				
120–135	34.8	10.09	14.4	7.31	64.7	6.60	6.07	0.653	0.504	0.149	0.199	0.312	0.304	0.014	0.318	101.9	0.035	11.2	0.054	17.3
135–150	36.4	11.09	14.9	7.32	60.7	6.66	6.19	0.673	0.541	0.132	0.196	0.308	0.308	0.010	0.318	103.2	0.031	10.1	0.052	16.9
150–165	37.9	12.25	15.1	7.33	61.5	6.66	6.32	0.753	0.602	0.151	0.206	0.308	0.314	0.008	0.322	104.5	0.034	11.0	0.052	16.9
165–180	37.9	12.25	16.0	7.34	62.1	6.40	6.43	0.762	0.631	0.131	0.208	0.300	0.301	0.006	0.309	102.4	0.029	9.7	0.054	18.0
Experiment 4; dog 1																				
100–115	33.2	9.31	14.0	7.29	70.6	5.66	5.89	0.657	0.534	0.123	0.207	0.357	0.344	0.026	0.370	103.6	0.030	8.4	0.059	16.5
115–130	32.4	9.47	14.4	7.29	74.6	9.13	5.99	0.706	0.596	0.110	0.214	0.380	0.370	0.018	0.388	102.1	0.027	7.1	0.065	17.1
130–145	33.1	9.84	14.7	7.29	73.2	9.40	6.07	0.720	0.613	0.107	0.214	0.380	0.366	0.015	0.381	100.3	0.026	6.8	0.066	17.4
145–160	34.1	10.23	14.5	7.29	73.2	11.00	6.21	0.749	0.642	0.107	0.221	0.357	0.353	0.011	0.364	102.0	0.026	7.3	0.064	17.9

were determined experimentally by titrating the urines electrometrically to the pH of the plasma. Substituting the quantities of buffer excreted, the observed pH values of the blood and urine, and the pK′ constants of the various buffers involved (phosphate 6.8, bicarbonate 6.1, and creatinine 4.97) into the Henderson-Hasselbalch equation, it was possible to calculate values for titratable acidity in the several ways noted in the remaining columns of the table. The titratable acidity of the urine was first calculated from the quantity of phosphate excreted and the observed pH of the urine. This calculated value approximates the observed titratable acidity, for the urinary buffer in these experiments was largely phosphate. However creatinine has some buffering capacity within this range of urinary pH. Since considerable quantities of creatinine were administered to measure glomerular filtration rate, its buffer value was significant although small. The sum of the titratable acidities calculated from the phosphate and creatinine excreted agrees closely with the observed titratable acidity. The maximum deviation is 4.5 per cent and the average agreement is within 2 per cent. The data therefore are sufficiently accurate to justify further analysis.

The quantity of titratable acid which could be excreted if the *phosphate reabsorption theory* were correct is rather low. It is apparent from the table that the total quantity of phosphate reabsorbed amounted to 0.100 to 0.151 mM. per min. If only dibasic phosphate were reabsorbed, the excess monobasic phosphate excreted in the urine would approximate $\frac{1}{5}$ of this amount. This excess monobasic phosphate would constitute the titratable acid of the urine. Accordingly this theory can account for only 6.8 to 11.6 per cent of the observed urinary acid.

The quantity of titratable acid which could be excreted if the *carbonic acid filtration theory* were correct is somewhat greater than that calculated on the basis of the phosphate reabsorption theory. The concentration of carbonic acid in the arterial plasma was calculated from the pH of the plasma and its CO_2 content. Multiplying this concentration by the rate of glomerular filtration gave the quantity of carbonic acid filtered each minute. If one assumes that no carbonic acid diffused back across the renal epithelium, i.e., that all the filtered carbonic acid appeared in the urine as titratable acid in the form of monobasic phosphate, the maximum acidity which this theory can explain amounts to only 16.5 to 26.5 per cent of the observed acidity.

These data demonstrate that both the phosphate reabsorption theory and the carbonic acid filtration theory are inadequate to account for the excretion of titratable acid under the conditions of our experiments. Since monobasic phosphate and carbonic acid are the only significant sources of acid in the glomerular filtrate, the high titratable acidities observed prove that acid must have been added by the renal tubules. In fact the data show that the sum of these two acids in the filtrate could account for only 24 to 37 per cent of the excreted acid. Therefore no less than $\frac{2}{3}$ to $\frac{3}{4}$ of the acid must have been added by the renal tubules. The tubular exchange of H^+ ions for Na^+ ions is but one of two possible mechanisms which can account for the acidification of the urine. Another possible mechanism is the tubular secretion of acid in molecular form. For reasons which will be detailed later we favor the ionic exchange hypothesis.

Acidification of the urine, if it is effected by a process of tubular exchange of H^+ ions for Na^+ ions, should be independent of the nature per se of the mechanism for excretion of the buffer. It should however be conditioned by the physico-chemical characteristics of the buffer. The more acid the pK′ of the buffer, the less readily should the tubular cells exchange their H^+ ions for Na^+ ions within the tubular lumen. Preliminary experiments with p-aminohippurate (pK′, 3.83) and creatinine (pK′, 4.97) indicated the verity of the above assumptions. That is, p-aminohippurate although filtered and secreted by the kidney is a poor renal buffer, because its buffering capacity is low within the physiological range of urine pH. Creatinine which is filtered through the glomeruli, but neither secreted nor reabsorbed, is a fair renal buffer, because approximately half of its buffering capacity is utilized in forming urine of pH 5.0. Creatinine as a buffer is peculiar in that it is a base. In blood at pH 7.4 it is almost completely in the form of the free base. In urine at pH 5.0 it is approximately one-half in the form of the acid salt of creatinine. We infer that the mixture of sodium chloride and free creatinine filtered through the glomeruli is transformed in part to creatinine hydrochloride in its passage through the renal tubules by the tubular exchange of H^+ ions for Na^+ ions.

Table 2 summarizes two representative experiments on acidotic dogs in which the major urinary buffer was creatinine. The plasma concentration of creatinine was raised to 180 to 219 mgm. per cent (16 to 19 mM. per l.) in order to cause the excretion of 1.22 to 1.59 mM. of creatinine per min. The degree of acidosis of the animal in experiment 5 was considerably greater than that in any other experiment in this series. The plasma phosphate concentration was very low in both experiments and as a consequence essentially no phosphate was excreted. The titratable acid of the urine, although considerably less than in the experiments with phosphate, was still high, varying between 0.099 and 0.146 m.eq. per min. This value is equivalent to the excretion of approximately 1500 to 2000 cc. of 0.1 N acid per day. It is apparent that the observed titratable acid agrees adequately with the acid calculated from the creatinine excreted and the urinary pH. The phosphate reabsorption theory is obviously incapable of explaining the acidity of the urine for essentially no phosphate was excreted. Furthermore since creatinine is present in the blood almost entirely in the form of free base, and since creatinine is not reabsorbed by the renal tubules, the concept of reabsorption of a basic component of a buffer mixture and excretion of an acid component is completely eliminated as an explanation of urinary acidification. The carbonic acid filtration theory is also incapable of explaining the observed urinary acid in these experiments. The inadequacy of this theory is somewhat less striking in experiment 6 than in the experiments in table 1, owing to the lesser excretion of titratable acid. However, the experiments presented in table 2 provide adequate confirmation of the general thesis that the elimination of acid in the urine is dependent on a tubular excretory process.

The rôle of renal carbonic anhydrase. The ultimate source of the H^+ ions which are added to the glomerular filtrate in its passage through the renal tubule must be carbonic acid. No other source of acid of sufficient magnitude (0.4 m.eq.

TABLE 2

Experiments on acidotic dogs designed to test critically the several theories of the nature of the renal mechanism for acidifying the urine

The major urinary buffer in these experiments was creatinine

TOTAL CONCURRENT TIME	ARTERIAL PLASMA CONCENTRATION				GLOMERULAR FILTRATION RATE	URINE		PHOSPHATE			CREATININE EXCRETED	TITRATABLE ACIDITY OF URINE								
												Observed	Calculated from							
	Creatinine	Phosphate	Total CO_2	pH		Flow	pH	Filtered	Excreted	Reabsorbed			Phosphate excreted	Creatinine excreted	Total buffer excreted		Phosphate reabsorption theory		Carbonic acid filtration theory	
Experiment 5; dog 1																				
min.	*mgm. %*	*mM./l*	*mM./l*		*cc./min.*	*cc./min.*		*mM./min.*	*mM./min.*	*mM./min.*	*mM./min.*	*mEq./min.*	*mEq./min.*	*mEq./min.*	*mEq./min.*	*% of observed*	*mEq./min.*	*% of observed*	*mEq./min.*	*% of observed*
120–135	180	0.23	6.89	7.25	78.7	16.5	5.84	0.018	0.001	0.017	1.25	0.146	0.001	0.143	0.144	98.6	0 000	0.1	0.036	24.7
135–150	187	0.23	7.05	7.27	74.2	18.1	5.86	0.017	0.001	0.016	1.22	0.136	0.001	0.134	0.135	99.2	0.000	0.1	0.033	24.3
150–165	204	0.23	6.91	7.30	73.9	20.9	5.94	0.017	0.001	0.016	1.32	0.121	0.001	0.122	0.123	101.7	0.000	0.1	0.030	24.8
165–180	219	0.23	6.61	7.30	67.2	20.0	6.02	0.016	0.001	0.015	1.30	0.099	0.001	0.102	0.103	104.0	0.000	0.1	0.027	27.3
Experiment 6; dog 5																				
100–115	196	0.62	14.2	7.33	89.7	10.7	6.02	0.056	0.000	0.056	1.55	0.125	0.000	0.121	0.121	96.8	0.000	0.0	0.072	57.6
115–130	199	0.56	14.9	7.34	85.4	11.3	6.00	0.048	0.000	0.048	1.50	0.127	0.000	0.123	0.123	96.8	0.000	0.0	0.068	53.5
130–145	209	0.58	15.0	7.35	85.0	12.1	6.00	0.049	0.000	0.049	1.57	0.126	0.000	0.129	0.129	102.4	0.000	0.0	0.068	54.0
145–160	214	0.60	15.3	7.37	84.1	11.7	6.03	0.050	0.000	0.050	1.59	0.118	0.000	0.121	0.121	102.5	0.000	0.0	0.066	55.9

per min.) is available to the kidney. The carbonic acid is no doubt derived both from the carbon dioxide of the peritubular blood and from that produced in the metabolism of the tubular cells. Wherever it is necessary to hydrate carbon dioxide to carbonic acid in large amounts, e.g., the red cells (9) the gastric mucosa (3) or the pancreas (17), one finds the enzyme carbonic anhydrase. According to Davenport and Wilhelmi (5) the cortex of the kidney contains this enzyme in high concentration. Sulfanilamide has been shown to be an inhibitor of carbonic anhydrase in vitro by Mann and Keilin (8), and to reduce the forma-

TABLE 3

The effect of sulfanilamide on the pH of the urine and the rate of excretion of titratable acid in acidotic dogs

The major urinary buffer in these experiments was phosphate

TOTAL CONCURRENT TIME	PLASMA CONCENTRATION				GLOMERULAR FILTRATION RATE	URINE		BUFFER EXCRETED		TITRATABLE ACID		
	Phosphate	Creatinine	Sulfanilamide	CO_2 combining power		Flow	pH	Phosphate	Creatinine	Observed	Total calculated	
Experiment 7; dog 5												
min.	*mM./l*	*mgm. %*	*mgm. %*	*vol. %*	*cc./min.*	*cc./min.*		*mM./min.*	*mM./min.*	*mEq./min.*	*mEq./min.*	*% of observed*
100–110	10.0	34.5	0.0	50.0	66.6	3.0	6.30	0.516	0.203	0.306	0.297	97.1
110–120	9.74	32.4	0.0	49.8	70.3	4.6	6.39	0.542	0.202	0.291	0.288	99.0
140–150	9.20	30.3	17.3	49.6	70.0	10.1	6.71	0.534	0.188	0.189	0.188	99.5
150–160	9.16	30.6	23.6	49.8	72.4	13.2	6.79	0.557	0.196	0.175	0.170	97.2
160–170	9.26	31.5	30.5	50.0	70.3	14.2	6.83	0.550	0.196	0.156	0.154	98.7
170–180	9.54	32.9	37.4	49.9	66.1	15.2	6.83	0.544	0.192	0.155	0.154	99.3
Experiment 8; dog 5												
105–115	9.35	29.8	0.0	47.7	69.8	6.1	5.55	0.535	0.184	0.435	0.431	99.1
115–125	9.48	29.6	0.0	47.9	74.3	8.8	5.57	0.593	0.194	0.466	0.467	100.2
125–135	9.67	29.6	0.0	48.0	74.8	11.3	5.61	0.619	0.196	0.478	0.471	98.6
155–165	9.90	31.1	54.0	45.7	76.9	16.5	6.67	0.687	0.211	0.234	0.243	103.8
165–175	10.1	32.0	66.0	47.5	75.6	16.7	6.77	0.700	0.214	0.199	0.207	104.0
175–185	10.7	33.1	78.4	48.5	71.6	15.6	6.81	0.679	0.210	0.177	0.186	105.0

tion of acid in the gastric mucosa in vivo by Davenport (4). Furthermore the administration of sulfanilamide in therapeutic doses frequently produces a mild acidosis clinically which is characterized by an increase in the pH of the urine (16). It is reasonable therefore to presume that carbonic anhydrase may play some rôle in the cellular processes involved in acid excretion (1, 6).

The nature of the rôle of renal carbonic anhydrase was investigated in a series of 8 experiments, representative examples of which are given in tables 3 and 4. In the experiments shown in table 3, phosphate was the major urinary buffer. The administration of sulfanilamide sharply reduced the observed titratable

acid of the urine. Since the quantity of buffer excreted was unaffected, the reduction of titratable acid was brought about by the increase in urinary pH. The increase in urinary pH was greatest in experiment 8; therefore the titratable acid decreased most in this experiment. However in both experiments considerable quantities of acid were eliminated at a time when the plasma concentration of sulfanilamide was very high. Since much lower concentrations of sulfanilamide than these inhibit the enzyme in vitro, it would appear that carbonic anhydrase, though it plays a rôle in acidification of the urine, is not essential to the process. Two additional experiments on other dogs gave identical results.

TABLE 4

The effect of sulfanilamide on the pH of the urine and the rate of excretion of titratable acid in acidotic dogs

The major urinary buffer in these experiments was creatinine

TOTAL CONCURRENT TIME	PLASMA CONCENTRATION: Phosphate	PLASMA CONCENTRATION: Creatinine	PLASMA CONCENTRATION: Sulfanilamide	PLASMA CONCENTRATION: CO_2 combining Power	GLOMERULAR FILTRATION RATE	URINE: Flow	URINE: pH	BUFFER EXCRETED: Phosphate	BUFFER EXCRETED: Creatinine	TITRATABLE ACID: Observed	TITRATABLE ACID: Total calculated	
min.	*mM./l.*	*mgm. %*	*mg. %*	*vol. %*	*cc./min.*	*cc./min.*		*mM./min.*	*mM./min.*	*mEq./min.*	*mEq./min.*	*% of observed*
Experiment 9; dog 5												
90–100	0.768	131	0.0	44.7	96.7	6.3	5.76	0.002	1.12	0.148	0.154	104.0
100–110	0.681	128	0.0	43.9	96.6	7.8	5.76	0.001	1.11	0.150	0.152	101.4
130–140	0.584	126	20.2	43.6	89.4	12.5	5.95	0.001	0.992	0.094	0.091	96.8
140–150	0.586	129	27.4	44.5	100.0	14.0	6.29	0.001	1.15	0.050	0.049	102.0
150–160	0.590	135	36.4	46.1	93.8	14.0	6.69	0.001	1.12	0.019	0.021	110.0
160–170	0.600	138	43.7	47.5	90.6	14.4	7.16	0.001	1.10	0.003	0.003	100.0
Experiment 10, dog 5												
90–100	0.442	145	0.0	31.6	68.6	6.6	5.54	0.001	0.878	0.180	0.182	101.2
100–110	0.380	140	0.0	31.8	73.2	7.2	5.54	0.001	0.907	0.180	0.188	104.5
110–120	0.332	135	0.0	32.0	71.9	8.0	5.54	0.001	0.856	0.181	0.177	97.8
130–140	0.252	126	35.1	33.0	75.1	11.6	5.79	0.001	0.834	0.110	0.108	98.2
140–150	0.242	119	38.4	34.0	76.6	9.6	5.82	0.001	0.805	0.100	0.098	98.0
150–160	0.252	133	45.6	35.0	75.4	10.1	5.92	0.001	0.889	0.087	0.087	100.0

In the experiments shown in table 4, creatinine was the major urinary buffer. In experiment 9, the administration of sulfanilamide reduced the excretion of titratable acid from 0.150 m.eq. per min. to essentially zero. In experiment 10, although the titratable acidity was significantly diminished by the administration of sulfanilamide, considerable quantities of acid were still eliminated. Two additional experiments were performed on two other dogs. One experiment duplicated experiment 9, and one was similar to experiment 10. Why sulfanilamide completely blocked the excretion of titratable acid in two experiments, and only diminished it in the other two experiments is not clear. The only essential

difference between the two groups of experiments was in the degree of acidosis. In both experiments in which the excretion of acid was blocked, the CO_2 combining power of the plasma was in a range of 43 to 48 vol. per cent. In both experiments in which the excretion of acid was merely diminished, the CO_2 combining power of the plasma was in a range of 30 to 35 vol. per cent. Despite these inconsistencies, the creatinine experiments confirm our general thesis that carbonic anhydrase plays a rôle in the cellular mechanism for acidifying the urine, but that it is not essential to that mechanism. The hydration of carbon dioxide to carbonic acid proceeds fairly rapidly in the absence of carbonic anhydrase. The enzyme merely speeds the process. It is possible to assume that the reduction in the rate of excretion of titratable acid when the enzyme is inhibited by sulfanilamide represents the effect of reducing the rate of hydration of carbon dioxide to its non-enzymatic level.[1] On the other hand, the enzyme may not be inhibited completely in vivo by the sulfanilamide concentrations attained in these experiments. Higher plasma concentrations of sulfanilamide cannot be attained safely, for in these experiments the animals were ataxic and exhibited spastic rigidity of the limbs and opisthotonus for as long as 12 to 48 hours following termination of the experiment. However none of the animals died or showed subsequent evidence of any renal damage.

Discussion. The data presented above are conclusive in showing that the quantity of acid excreted in the urine may far exceed the quantity of acid filtered through the glomeruli. Therefore the urine must be acidified by some renal tubular mechanism which adds H^+ ions to the glomerular filtrate. The only intracellular source for the large quantity of H^+ ions required is carbonic acid, formed within the tubule cells by the hydration of carbon dioxide. Supporting this concept are the experiments with sulfanilamide. The inhibition of carbonic anhydrase by this drug and the consequent retardation of the rate of hydration of carbon dioxide impair the ability of the renal tubules to acidify the filtrate.

The tubular mechanism for the addition of H^+ ions to the filtrate has been related to a process of ionic exchange. Actually there is a variety of ways in which this might be effected. The simplest and to us the most acceptable concept would be a direct exchange of H^+ ions for Na^+ ions, thereby accomplishing in a single process the desired end, namely, the excretion of acid and the conservation of base. The various salts present in the filtrate are in ionized form, and it seems reasonable to assume that the kidney operates on ions, not molecular species, within the limitations imposed by the necessity for maintaining ionic electroneutrality. This latter condition is met by the exchange of H^+ ions for Na^+ ions across the tubular epithelium. It is possible however to postulate various indirect methods by which this ionic exchange might be carried out. For example, either HCl or H_2CO_3 might be secreted by the tubule cells into the lumen. These acids would react with urinary buffers with the production of NaCl or $NaHCO_3$. We have observed experimentally that large quantities of titratable acid may be eliminated as NaH_2PO_4, yet the urine may be essentially chloride and bicarbonate free. Therefore if either HCl or H_2CO_3 is secreted by

[1] See addendum.

the tubule cells, the NaCl or $NaHCO_3$ formed must be subsequently reabsorbed from the urine. It will be noted that the end result of such a mechanism is to exchange H^+ ions for Na^+ ions, although the details of the process are different from those postulated above. At present it does not appear possible to obtain conclusive evidence as to whether direct or indirect processes accomplish this ionic exchange.

Our concepts of the nature of the cellular mechanism are presented in diagrammatic form in figure 2 which illustrates a single renal cell from that part of the distal tubule which is concerned with acidification of the urine. The luminal border of this cell is in contact with urine; the opposite border is in contact with interstitial fluid which is in diffusion equilibrium with the peritubular blood. H^+ ions dissociated within the cell are exchanged across the luminal border for Na^+ ions in the tubular urine. There appears to be a maximum concentration

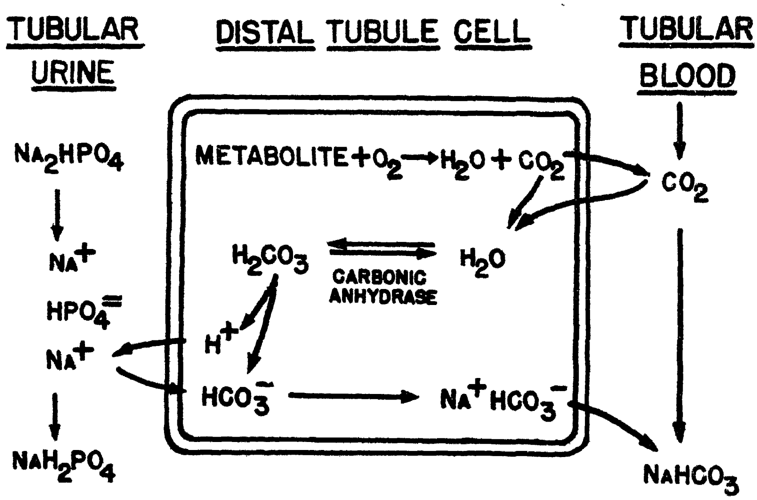

Fig. 2. Diagrammatic representation of the nature of the renal cellular mechanism for acidifying the urine. A single cell from the distal segment of the renal tubule is illustrated.

gradient for H^+ ions which can be established across this membrane. In urine of maximum acidity, pH 4.8, the concentration of H^+ ions is approximately 400 times greater than in the blood. The actual gradient across the luminal membrane is unknown, for the pH of the interior of the tubular cell has not been measured. The gradient between urine and blood is that established across both membranes. When the urinary content of buffer is raised, the quantity of H^+ ions which can be transferred is much increased, for the concentration gradient against which they are transferred may be considerably reduced. For example in table 1, experiment 3, period 4, 0.300 m.eq. of H^+ ion per min. was transferred against a gradient of only 8:1. In the absence of buffer, despite a maximum gradient of 400:1, negligible quantities of H^+ ion are transferred; i.e., the titratable acidity is very low. Obviously the pK′ of the buffer affects the process of transfer of H^+ ions: the lower the pK′, the greater the con-

centration gradient which must be attained in order to transfer a given quantity of H^+ ions. Phosphate, pK′ 6.8, is a much more effective urinary buffer than is creatinine, pK′ 4.97.

The intracellular production of large quantities of H^+ ions is dependent upon the rapid hydration of carbon dioxide to carbonic acid. The cellular sources of carbon dioxide are two: it is produced by cellular metabolic processes and is derived from the postglomerular blood. Carbonic anhydrase, by speeding the hydration of carbon dioxide, increases the rate of production of H^+ ions in the tubular cells. Our experiments with sulfanilamide indicate, however, that carbonic anhydrase may not be essential for the elimination of acid in the urine. The hydration of carbon dioxide proceeds with moderate rapidity in vitro in the absence of enzyme. The non-enzymatic rate of hydration is probably sufficient to explain the residual titratable acidity in those experiments in which the enzyme was presumably inhibited by sulfanilamide.

HCO_3^- ions formed by the dissociation of carbonic acid are absorbed into the renal tubular blood in combination with equivalent numbers of Na^+ ions. The mechanism thus accomplishes the excretion of acid and the retention of base by a single fundamental process based primarily on the exchange of H^+ ions for Na^+ ions.

Acidification of the urine is a process which requires the expenditure of energy. If energy were applied to effect the movement of one ion type at any one of several points, the rest of the system might function spontaneously. Thus if H^+ ions were actively pumped out of the cell into the tubular lumen, Na^+ ions would have to move in the reverse direction to maintain ionic balance. The high cellular concentration of sodium bicarbonate would set up a diffusion gradient between the interior of the cell and the tubular blood. Passage of bicarbonate into the blood would be determined by diffusion forces. Presumably selective impermeability of the luminal membrane in the reverse direction would prevent diffusion back into the urine. On the other hand, if sodium bicarbonate were actively pumped out of the cell into the tubular blood, all other processes might occur by diffusion. Or if energy were utilized to force Na^+ ions into the cell, diffusion forces might account for the movements of the remaining components. We know of no valid reason for choosing between these several possibilities. However no system dependent entirely on diffusion processes could account for the continued movement of acid from blood to urine. Energy must be expended at some point.

While the evidence is conclusive that acidification of the urine is effected in large part by some renal cellular mechanism, do the *phosphate reabsorptive mechanism* and the *carbonic acid filtration mechanism* contribute to the excretion of acid? From evidence on the dog and amphibian kidney, the phosphate reabsorptive concept may be entirely ruled out. Walker and Hudson (20) have shown in the frog that phosphate is reabsorbed in the proximal tubule, whereas Montgomery and Pierce (10) have shown that acidification of the urine is effected in a very localized segment of the distal tubule. Since the pH of the tubular urine does not change in the proximal tubule where phosphate is reabsorbed,

dibasic and monobasic phosphate must both be reabsorbed, and furthermore they must be reabsorbed in the same proportions as they exist in the blood. Hence reabsorption of phosphate cannot contribute to the acidity of the urine. The evidence on the dog confirms this view indirectly. The maximum reabsorptive capacity for phosphate was shown by Pitts and Alexander (13) to be the same in normal and in acidotic dogs. The normal animals excreted small quantities of titratable acid, the acidotic animals excreted large amounts. Therefore the excretion of acid could not be determined in any way by the reabsorption of phosphate.

In general the same line of argument can be used to negate any appreciable contribution of a carbonic acid filtration mechanism. Thus in the frog water is reabsorbed in the proximal tubule, the total osmotic pressure of the fluid in the lumen remains constant (21), therefore bicarbonate is reabsorbed in proportion to the reabsorption of water. Since pH does not change in the proximal tubule, carbonic acid or more properly dissolved carbon dioxide must diffuse back in proportion to bicarbonate. It cannot therefore contribute appreciably to the acidity of the urine. In the dog it is presumed that obligatory water reabsorption in the proximal tubule reduces the volume of fluid to $\frac{1}{5}$ of that in the original glomerular filtrate (15). If the urine is acidified in the distal tubule, it is obvious that $\frac{4}{5}$ of the dissolved carbon dioxide must have diffused back in the proximal tubule before the segment was reached in which acidification is effected. A more indirect line of evidence likewise argues against the carbonic acid filtration concept. Carbon dioxide in an aqueous medium is largely in the form of the dissolved gas and only a small proportion is in the form of carbonic acid. Cell membranes in a moist state are uniformly permeable to carbon dioxide. It is highly improbable that the renal epithelium is so very different from other cell membranes as to be completely impermeable to carbon dioxide. Therefore one would expect some approach to equilibrium conditions to be maintained all along the renal tubule between the urine and the renal venous blood. Hence carbonic acid in the filtrate could contribute little to urinary acidity. Occasional high tensions of carbon dioxide in bladder urine can be explained in two ways: 1, the result of the mixture of acid and alkaline urine in the bladder; 2, the result of the delayed dehydration of carbonic acid to carbon dioxide in the tubular urine, which is of course devoid of carbonic anhydrase.

It is instructive to relate these experiments on the dog to observations made on man in conditions of clinical acidosis. In severe diabetic acidosis the titratable acidity of the urine may increase to as much as 1500 cc. of 0.1 N acid per day (12). Much higher rates of acid elimination have been attained in our experiments on dogs. For short periods rates equivalent to 6000 cc. or more of 0.1 N acid per day have been observed during the infusion of phosphate. This quantitative difference is certainly related to the physicochemical properties of the buffer excreted and not to a greater effectiveness of the acid eliminating mechanism in the dog. The major buffer acid in the urine of the diabetic is B-hydroxybutyric, the pK′ of which is 4.7. It is therefore somewhat less effective as a urinary buffer than is creatinine, and far less effective than is phosphate.

Rates of acid elimination in the dog during the infusion of creatinine are more nearly comparable to those observed in the diabetic, namely, rates equivalent to 1500 to 2000 cc. of 0.1 N acid per day. In chronic diffuse glomerulonephritis the capacity of the kidney to eliminate acid is reduced (12). Accordingly, acidosis develops as a result of the daily dietary intake of an excess of potential acid forming substances. In terminal nephritis the titratable acid of the urine falls to as low as 5 or 10 cc. of 0.1 N acid per day, and the CO_2 combining power and pH of the blood fall as the alkali reserve of the body is progressively exhausted.

SUMMARY

In a series of 18 experiments on 4 trained female dogs rendered acidotic by the daily administration of hydrochloric acid by mouth, the following facts have been observed, and the following inferences drawn from these facts.

1. When the animals were caused to excrete large quantities of buffer in the urine by the intravenous administration of infusions containing phosphate, as much as 0.380 m.eq. per min. of titratable acid was excreted. When the infusions contained creatinine in place of phosphate as much as 0.150 m.eq. per min. of titratable acid was excreted. In both series of experiments the quantity of acid excreted far exceeded the quantity of acid filtered through the glomeruli. Accordingly it has been demonstrated that a cellular mechanism must add acid to the glomerular filtrate as it passes through the renal tubules.
2. It is suggested that this addition of acid is effected by the direct exchange of H^+ ions formed within the renal tubular cells for Na^+ ions present in the tubular urine. This process probably occurs in the distal segment of the tubule.
3. The intracellular source of H^+ ions is carbonic acid formed by the hydration of carbon dioxide produced metabolically within the renal cells and derived by them from the postglomerular blood.
4. Renal carbonic anhydrase, by increasing the rate of hydration of carbon dioxide and hence the rate of formation of H^+ ions, increases the rate of acid elimination. The enzyme however is probably not essential, for when it is inhibited by sulfanilamide, acid elimination continues although at a slower rate.
5. The Na^+ ions which enter the renal tubular cells in exchange for H^+ ions are absorbed into the peritubular blood as sodium bicarbonate.
6. The pK′ of the urinary buffer influences the quantity of acid excreted: the lower the pK′, the less acid excreted. Accordingly phosphate is a more effective urinary buffer than creatinine, and creatinine is more effective than is p-aminohippurate.
7. The relation of these findings to the behavior of the kidney in clinical acidosis is briefly discussed.

ADDENDUM

Since this paper has been submitted, Davenport (J. Biol. Chem. **158**: 567, 1945) has published a study of the inhibition of carbonic anhydrase by sulfanilamide. In concentrations of 25 to 50 mg. per cent, sulfanilamide inhibited 99.93 to 99.97 per cent of the enzyme present in red cells. However the remaining 0.03 to 0.07 per cent of the enzyme was capable of hydrating carbon dioxide at a rate from 17.5 to 34.2 per cent of the control. Davenport

emphasizes the fact that the enzyme is present in tissues in tremendous excess, and that complete inhibition of function of the enzyme could not be expected in previous studies on gastric secretion, pancreatic secretion, and renal acid excretion at the relatively low plasma concentrations of sulfanilamide employed.

In our experiments plasma concentrations of sulfanilamide have ranged between 17 and 78 mg. per cent. We may therefore expect that 99.9^{+} per cent of the enzyme has been inhibited. Since we have loaded our animals with buffer and hence have increased the demands on the carbonic anhydrase system tremendously, the remaining active enzyme (probably less than 0.1 per cent) is less than adequate to maintain the control rate of acid elimination. However the residual excretion of titratable acid which we have noted (from 2 to 50 per cent of the control) may be most logically assigned to the activity of this residual uninhibited fraction of the enzyme rather than to the non-enzymic hydration of carbon dioxide as noted in the text. The general agreement of our figures on reduction of function with those published by Davenport strengthens our conviction that renal carbonic anhydrase is an important component in the mechanism for excretion of titratable acid.

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THE SURVIVAL TIMES OF EVISCERATED RATS AS INFLUENCED BY THE CONTINUOUS INTRAVENOUS ADMINISTRATION OF A SOLUTION OF SODIUM CHLORIDE

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Laboratory animals usually die within a few hours following the removal of the liver unless glucose is administered. We have been able to prolong the survival of eviscerated rats by the continuous intravenous injection of a solution of 0.9 per cent sodium chloride.

METHODS. Male rats of the Sprague-Dawley strain were used. The diet was Purina Dog Chow. When the rats reached a weight of 180 to 190 grams the inferior vena cava was ligated between the liver and kidneys in order to cause the development of a collateral circulation. Asepsis was preserved during this operation. When the animals reached a weight of 250 grams they were eviscerated by the procedure described by Ingle and Griffith (1). The animals were not fasted. All of the intra-abdominal organs were removed except the adrenals and the kidneys.

Intravenous infusions were made by a continuous injection apparatus which delivered fluid from each of two syringes at the rate of 20 cc. in 24 hours. The infusions were started within 20 minutes following the removal of the liver. The apparatus was powered by a synchronous motor and the reduction of motion was achieved by a precision built system of gears and levers so that an exact control of the rate of injection was attained. The injections were made into the saphenous vein of the right hind leg.

The analyses of blood glucose were made by the method of Miller and Van Slyke (2). Blood samples were taken from the tail just prior to the beginning of infusions and 2, 6 and 24 hours later in those animals which survived. In a few of the animals blood samples were taken at the time of death.

Three experiments were carried out simultaneously. Each experimental group consisted of 12 animals. In experiment 1, the animals were operated under ether anesthesia and were observed without further treatment until death. In experiment 2, the animals were operated under barbiturate anesthesia (18 mgm. of cyclopentenyl-allyl-barbituric acid sodium by intraperitoneal injection), were secured in a supine position on an animal board and were observed without further treatment until death. In experiment 3, the animals were operated under barbiturate anesthesia, were secured in a supine position on an animal board and were given intravenous infusions of a solution of 0.9 per cent sodium chloride until death.

RESULTS. The times of survival and the values for blood glucose of indivdual animals are summarized in table 1. The animals which were operated under ether anesthesia and left without additional treatment showed a range in survival

times of 5 to 22 hours with an average of 16.6 hours. The animals which were eviscerated under barbiturate anesthesia and did not receive additional treatment showed a range in survival times of 7 to 27.5 hours with an average of 17.3

TABLE 1

Values for blood glucose in eviscerated rats

EXPERIMENTAL CONDITION	HOURS OF INFUSION				FINAL	HOURS SURVIVAL
	0	2	6	24		
Barbiturate anesthesia and intravenous saline	64.5	47.5	46.5	34.5	24.0	30.0
	84.0	47.7	37.5			20.0
	67.5	28.5	33.0	31.0		27.0
	94.5	45.0	42.0	24.0		27.0
	79.5	34.0	28.5	28.0		28.0
	76.5	37.5	35.5	39.0	41.5	30.5
	63.0	30.5	37.5	20.5		25.0
	84.0	47.5	42.0	34.5	30.0	31.5
	67.5	34.0	28.5	31.5		24.0
	70.5	53.5	21.0	31.0		25.5
	75.0	42.0	24.0	18.0		23.0
	69.0	30.0	24.0	21.0		25.0
Barbiturate anesthesia No saline	60.0	28.0	46.0			16–18
	72.0	39.0	27.0			9–11
	73.0	31.5	25.0			16–18
	70.0	29.5	43.5			17–19
	86.5	41.5	34.0	41.5	51.0	27.5
	81.0	40.0	27.0			20
	81.0	40.5	44.5		33.0	23
	73.5	28.5	27.0			16
	71.0	25.5	20.5			18
	77.0	39.0	29.5			15–17
	61.0	45.5	34.5			17–19
	97.5	34.5	25.5			7
Ether anesthesia. No saline	106.0	33.0	32.0		43.5	22
	98.0	34.5	24.0			15–17
	82.0	20.5	31.5			18
	79.5	32.5	28.5			20
	80.5	30.0	31.0			17.5
	73.5	24.0			21.0	5.5
	70.5	43.5	25.5			18
	75.0	36.0			31.0	5
	81.0	34.5	27.0			16–17.5
	92.3	36.8	37.5		46.0	22
	87.8	35.3	30.0			18
	93.8	48.8	51.8			20

hours. Similar animals which were infused with a solution of sodium chloride showed a range in survival times of 20 to 31.5 hours with an average of 26.4 hours.

The animals which were eviscerated under ether anesthesia tended to have

higher initial values for blood glucose than the animals which were operated under barbiturate anesthesia. The blood glucose values tended to fall less rapidly in the animals which were infused with saline although after six hours the values were similar in each of the three groups. The greatest decrease occurred within the first two hours following operation. Among the animals which were infused with saline the average initial value was 74.6 mgm. per 100 cc. and at periods of 2, 6 and 24 hours later the average glucose concentrations had been reduced to 39.8, 33.3 and 28.4, respectively. There were four animals in which the blood glucose value at 24 hours was higher than either or both the two and six hour levels.

DISCUSSION. The average survival of eviscerated rats was significantly prolonged by the continuous intravenous injection of physiological saline. Roberts, Samuels and Reinecke (3) reported that fat-fed rats survived evisceration longer than carbohydrate-fed rats. Reinecke and Roberts (4) found that fasting rats survived evisceration longer than animals fed Purina Fox Chow up to the time of operation. The diet used in the present experiments was high in its content of carbohydrate and the animals were not fasted prior to operation. In the experiments of Reinecke and Roberts (4) the survival times of fed animals were within the range of survival times of similarly tested animals (expt. 1) in the present study.

The reason for the beneficial effect of the continuous intravenous injection of saline is not known to us. It may have to do with a better maintenance of circulatory mechanisms and be related to the observations of Russell, Long and Engel (5) and of Reinecke (6) that the withdrawal of blood from the eviscerated rat increases the rate at which the blood glucose falls and shortens the time of survival.

It is clear from these data and the results of others (3, 4, 6) that the rate of fall of blood glucose is negatively accelerated in the eviscerated non-nephrectomized rat. Roberts, Samuels and Reinecke (3) observed a sharp decline in oxygen consumption after evisceration which leveled off to about 25 per cent that of the intact animal within two or three hours. There may have been other adaptive mechanisms leading to an almost total cessation of the removal of glucose from blood or it may have been supplied to the blood at a rate which equalled its loss either from tissue stores or by gluconeogenesis in tissues other than the liver (6). The few instances in which the amount of glucose in the blood appeared to rise may have been an artifact of sampling or analysis or it may have been due to the accumulation of non-fermentable reducing substances (3).

SUMMARY

Male rats were eviscerated at a body weight of 250 grams. The kidneys and adrenals were not removed. Animals operated under barbiturate anesthesia and infused intravenously with a solution of 0.9 per cent sodium chloride at a rate of 20 cc. per 24 hours survived from 20 to 31.5 hours with an average of 26.4 hours. A control group which was treated in an identical manner except for

the administration of saline lived from 7 to 27.5 hours with an average of 17.3 hours. A second control group which was operated under ether anesthesia survived from 5 to 22 hours with an average of 16.6 hours.

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THE INFLUENCE OF EXCITATION OF MUSCLE PAIN RECEPTORS ON REFLEXES OF THE DECEREBRATE CAT[1]

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Previous investigations (Thompson and Gellhorn, 1945) have shown that intramuscular injection of hypertonic NaCl solution alters significantly the flexor reflex and the knee jerk in the anesthetized cat. The flexor reflex was diminished by contralateral injection of NaCl, and increased by ipsilateral injection. With respect to the knee jerk, in general the reverse result was found, but it was also observed that in some instances the knee jerk was inhibited by a contralateral injection of NaCl. Pupillary dilatation and vocalization resulting from intramuscular injection of hypertonic solutions (Gellhorn and Thompson, 1944) in animal experiments as well as the observations of Lewis (1942) in man indicated that pain fibers were excited under these conditions. The present paper attempts to extend these observations by the study of the influence of muscle pain impulses on the contralateral extensor reflex of the decerebrate cat. This study was undertaken for several reasons: first, in order to determine whether the crossed extensor reflex reacts in response to pain impulses in a manner similar to that described earlier for the knee jerk; second, to evaluate the influence of the increased muscle tone which characterizes the decerebrate animal on the effects of pain impulses; third, to study the effect of pain impulses on reciprocal innervation.

METHOD. The report is based on experiments performed on 12 cats which were decerebrated with Sherrington's decerebrator under ether anesthesia. The quadriceps muscles of one or both legs were prepared and their tension recorded by means of isometric levers. Ipsilateral and contralateral[3] nerves (sciatic, saphenous, or peroneal) were isolated and their central ends were stimulated by means of the Harvard inductorium with 3 volts in the primary. Mechanical devices served to maintain the stimulation of the nerve for a given period at constant intervals. In some experiments rhythmical condenser discharges were used (stimulator of C. Goodwin). Small amounts of hypertonic NaCl solution were injected into various muscles.

RESULTS. I. *The effect of ipsilateral and contralateral stimulation of muscle pain fibers on the contralateral extensor reflex in cats with low extensor tonus.* It was found that ipsilateral intramuscular injection of 15 per cent NaCl (0.1 to 0.5 cc.) invariably diminished the reflex contraction[4] whereas the effect of con-

[1] Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

[2] Deceased January 31, 1945.

[3] These terms are used with respect to the recorded muscle.

[4] Injection into various muscles of the same side had similar effects.

tralateral injection of NaCl on the extensor reflex was variable. In four out of ten experiments the reflex was greatly diminished, in five it was increased, and in one an initial decrease was followed by a later increase. The results were similar to those described for the knee jerk by Thompson and Gellhorn. Here also it was found that ipsilateral NaCl injection regularly diminished the reflex response whereas contralateral injection either diminished or increased the knee jerk. The experiments differed quantitatively from those reported earlier inasmuch as in decerebrate preparation, apparently due to the absence of anesthesia, the stimulation of the pain fibers was more effective in degree and duration.

Figure 1 illustrates the inhibitory effect exerted by ipsilateral stimulation of muscle pain fibers on the extensor reflex. In figure 1a the reflex response consists in an initial phase in which the greatest tension is reached almost immediately, followed by a second phase in which a moderate tension is maintained during the duration of the stimulus. There is only a slight after-discharge which is followed by spontaneous movements. The latter were initiated by previous injection of strychnine and followed the reflex response in regular intervals (x in fig. 1a). After the injection of 0.5 cc. 15 per cent NaCl into the ipsilateral hamstrings the initial response is reduced by $\frac{1}{3}$, the second phase of the reflex contraction is almost completely abolished, and the spontaneous strychnine contractions likewise disappear. The effect is not reversible but a slight increase in the intensity of reflex stimulation restores the reflex to approximately its original intensity. Figure 1b shows a similar experiment obtained on the same preparation some time later in which the effect of ipsilateral NaCl injection is reversible. Here again the reflex tension decreases markedly as a result of ipsilateral muscle pain impulses.

Figure 2 shows an experiment in which under control conditions the extensor reflex is characterized by a slight rebound effect and a marked after-discharge. Under the influence of ipsilaterally injected NaCl the reflex contraction during the stimulation of the sciatic disappears almost completely at first (no. 5), is regained to a slight extent temporarily at no. 6, and is replaced by a slight loss in tone in the following experiments (7–9). The effect is not reversible but on increasing the intensity of the stimulus (no. 16) a similar reflex, characterized not only by a reflex tension maintained during the period of stimulation but also by rebound and after-discharge, is obtained again.

In those cases in which contralateral injection of NaCl had an inhibitory effect on the reflex response the effects were similar to those just described and are therefore not further illustrated but a mixed and an excitatory effect resulting from contralateral NaCl injection are illustrated in figure 3. A distinct and moderate decrease in the intensity of the reflex response occurs at first in figure 3a. This, several minutes later, is followed by an increased extensor reflex. The reflex response begins in this experiment under control conditions with a considerable latent period and a gradual development of tension which reaches its peak at the end of the period of stimulation. There is a quick relaxation and no after-discharge. In the experiments 11 to 15 which follow the depressive phase of the NaCl effect there is immediately an excitatory phase at the onset of the

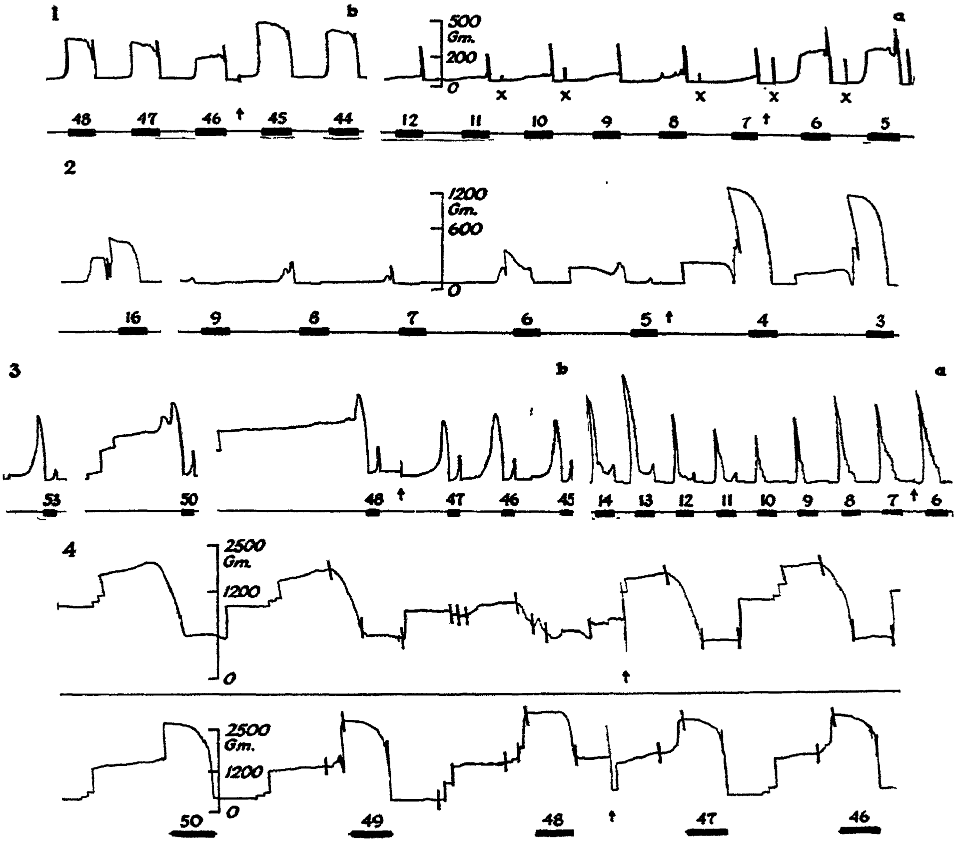


Fig. 1a. Cat, ♀,1.8 kgm., three-tenths of a centimeter strychnine sulphate, 1 per cent prior to experiment. Effect of 0.5 15 per cent NaCl injected into the (right) hamstrings at the arrow (45 seconds before no. 7 is recorded) on the right quadriceps reflex. Stimulation of contralateral (left) sciatic for three seconds at intervals of two minutes. Harvard inductorium 3 V in the primary; coil distance (c.d.) 13 cm. All records are from right to left.

Fig. 1b. Same preparation; 0.5 cc. 15 per cent NaCl injected into ipsilateral (right) hamstrings 45 seconds before no. 46 (arrow). Left sciatic stimulated at 11 cm. c.d.

Fig. 2. Injection of 0.5 cc. 15 per cent NaCl 45 seconds before no. 5 (arrow) into ipsilateral (right) hamstrings. Right quadriceps muscle recorded. Left sciatic stimulated for three seconds at 2 minute intervals at c.d. 13 cm. No. 16 several minutes later, sciatic stimulated at c.d. 11 cm.

Fig. 3a. Injection of 0.2 cc. 15 per cent NaCl before no. 7 (arrow) into contralateral (left) hamstrings. Right quadriceps muscle recorded. Left sciatic stimulated at 13 cm. and 80°; duration of stimulation 2½ seconds. Interval 1½ minutes.

Fig. 3b. Same preparation; 0.5 cc. 15 per cent NaCl injected into contralateral (left) hamstrings before no. 48 (arrow); c.d. 13 cm. 60°.

Fig. 4. Double quadriceps preparation of decerebrate cat. Upper record, right quadriceps; lower record, left quadriceps. Signal records indicate stimulation of right sciatic, 13 cm., 80°; duration of stimulation 2½ seconds, intervals two minutes; 0.1 cc. 15 per cent NaCl is injected into right hamstrings 45 seconds before no. 48 (arrow). The black lines crossing the tension curves in figures 4 to 7 indicate simultaneity.

stimulus, a low tension is maintained during stimulation and at the end of the period of stimulation a very marked rebound occurs in which a much higher tension is reached than under the control conditions which preceded the injection of NaCl.

An example of an excitatory effect of contralateral NaCl injection is shown in figure 3b. In this experiment the reflex response consists under control conditions of an initial reflex contraction which gradually subsides during the period of stimulation but is followed by a marked rebound and after-discharge. Contralateral NaCl injection increases the rebound in intensity and greatly prolongs the after-discharge. The experiment is reversible as seen by the record obtained several minutes later (no. 53). On closer inspection of these experiments it was found that excitatory effects resulting from the contralateral NaCl injection were associated with a slight increase in tone. Assuming that variation in tone might provide the clue for the variability of the extensor reflex in response to contralateral excitation of muscle pain fibers a series of experiments was performed on decerebrate cats which had a very high degree of rigidity.

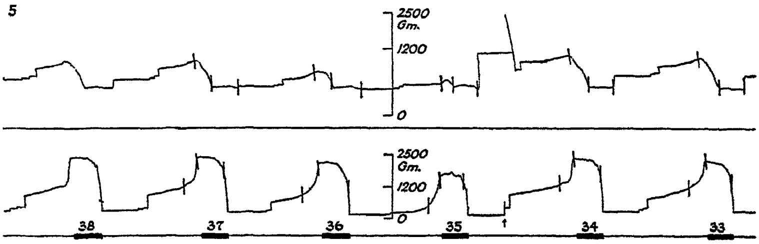
II. *The effect of muscle pain impulses on the muscle tone in decerebrate rigidity and on excitatory and inhibitory reflex effects.* Eight experiments were performed on cats with extreme rigidity in which the tension of both quadriceps muscles was recorded and one of the sciatics was stimulated.[5] Under these conditions reciprocal innervation occurred, stimulation of the sciatic causing an increased reflex tension in the contralateral quadriceps but an inhibition of the ipsilateral extensor muscles. Muscle tone and reflex response were altered in a uniform manner as a result of afferent pain impulses. The tone of the quadriceps was increased on the contralateral side (with respect to the site of NaCl injection) but decreased on the ipsilateral side. As to the effect of afferent pain impulses induced by intramuscular injection of hypertonic NaCl solution on reflex excitation and inhibition the experiments may be divided into two groups. In the first group NaCl injection and sciatic stimulation were performed on the same extremity whereas in the second group the afferent muscle pain impulses originated in the side contralateral to the sciatic which was stimulated. Figure 4 illustrates the action of NaCl on the reflex in experiments belonging to the first group. Under the influence of NaCl the tone of the opposite quadriceps (left) increases greatly (lower half of fig. 4). Sciatic stimulation (right) results in greater reflex tension of the left quadriceps than under control conditions. The reflex tension is increased by more than 400 grams[6] in the first reflex elicited after an NaCl injection. However, the effect is reversible and the reflex tension returns in subsequent tests to control values. Still greater are the changes observed in the upper half of figure 4 in which the tone of the right quadriceps is reflexly inhibited since it is recorded from the side ipsilateral to that of the stimulated sciatic. It is seen that NaCl causes not only a diminution in the tone of

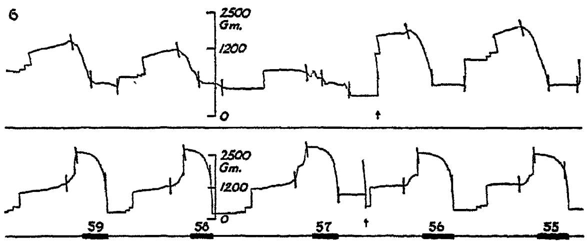
[5] Since the tension of both quadriceps muscles is recorded the terms ipsilateral and contralateral refer to the sciatic nerve which is stimulated.

[6] The exact increase was not determined because the lever touched the signal magnet above it at this point.

the quadriceps but also diminishes the reflex inhibition of this tone under conditions of ipsilateral sciatic stimulation. The after-discharge following stimulation of the sciatic is likewise diminished. Both effects are reversible.

Figure 5 may serve as a representative of the second group of experiments in which NaCl injection and sciatic stimulation were performed on opposite sides.





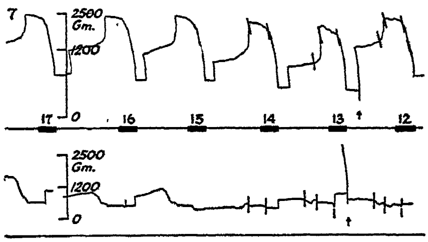


Fig. 5. Same preparation. Sciatic stimulation as above; 0.1 cc. 15 per cent NaCl injected into left hamstrings 45 seconds before no. 35 (arrow).

Fig. 6. Double quadriceps preparation. Right above; left below. Stimulation of right sciatic (c.d. 13 cm. 80°) for 2½ seconds; intervals two minutes. Injection of 0.2 cc. 15 per cent NaCl into the right hamstrings 45 seconds before no. 57 (arrow). Reciprocal innervation is temporarily abolished and changed into co-contraction.

Fig. 7. Double quadriceps preparation. Stimulation of left sciatic; intervals as in figure 6; 0.1 cc. 15 per cent NaCl injected 45 seconds before no. 13 (arrow) into right hamstrings. Co-contraction temporarily changed under the influence of afferent pain impulses into reciprocal innervation. Several minutes later nos. 16 and 17 are recorded. In no. 17 the muscle is brought under an increased initial tension. This caused a reflex reversal.

The upper half of figure 5 shows the inhibitory effect of the stimulation of the right sciatic on the ipsilateral (right) quadriceps, whereas the lower half shows the excitatory effects on the contralateral (left) quadriceps. Following the injection of 0.1 cc. 15 per cent NaCl into the left hamstrings the tone of the right quadriceps (upper half of fig. 5) greatly increases and its response to ipsilateral stimu-

lation of the sciatic is quantitatively altered. Reflex inhibition is markedly increased and after-discharge and rebound, which were prominent prior to the injection of NaCl, are almost completely abolished. However, the effect is reversible and the original response including after-discharge and rebound is gradually restored.

The lower half of figure 5 illustrates the behavior of the contralateral extensor reflex under the influence of ipsilateral injection of NaCl. The tone decreases slightly and the reflex tension is greatly lowered. A diminution in the excitatory reflex effect is further illustrated by the fact that the after-discharge is likewise diminished. All effects are reversible and can be repeated several times in the same animal.

Summarizing these results it may be stated that under the influence of very small quantities (0.1 cc.) of hypertonic NaCl solution, which sets up afferent pain impulses in muscle, both excitatory and inhibitory processes are quantitatively altered. This is shown in the study of the tone of the decerebrate animal as well as in the alteration of reflex responses. It is found that:

1. Afferent pain impulses increase the tone of the quadriceps muscle contralaterally and inhibit it ipsilaterally.

2. The contralateral extensor reflex is increased when as a result of pain impulses the tone is increased. This augmentation of response pertains not only to reflex tension developed during stimulation but also to the excitatory processes which follow cessation of stimulation (rebound and after-discharge). However, if as a result of afferent pain impulses the tone of the muscles in decerebrate rigidity is decreased, the excitatory processes (reflex tension, rebound, and after-discharge) are likewise diminished.

3. Reflex inhibition due to ipsilateral stimulation of the sciatic is increased when the tone is increased and diminished when the tone is lessened as a result of centripetal pain impulses.

III. *Pain impulses and reciprocal innervations.* The experiments described in the preceding section gave evidence of a marked alteration in the reflex response as a result of pain impulses originating in muscle. It was found that, in general, the effect of these impulses consisted in a quantitative, but not in a qualitative alteration of excitatory and inhibitory processes. However, close inspection of some of the records reveals changes which are even more fundamental. Thus, it is seen in figure 2 that as a result of pain impulses, excitation is reversed into inhibition, in numbers 7 to 9 during the stimulation of the sciatic. To be sure this inhibition is only very slight but this is thought to be due to the fact that the tone in this preparation is very low. This interpretation is supported by observations on reflex reversals in cats with marked decerebrate rigidity.

Figure 6 illustrates an experiment with a double quadriceps preparation in which prior to the elicitation of pain impulses stimulation of the sciatic results in a typical contralateral reflex extension and inhibition of the ipsilateral quadriceps. Injection of NaCl into a muscle ipsilateral to the sciatic which is stimulated, results in an increased tone of that quadriceps which reacts with reflex

excitation and a decrease in tone of the muscle which under control conditions shows inhibition on stimulation of the sciatic nerve. The former responds under the influence of pain impulses, as was described before, with a greater tension. The latter, however, instead of showing a lessened inhibition which was described as the typical result for such conditions, shows no inhibition but an increase in tension, i.e., an excitation as a result of sciatic stimulation (no. 57). At the next test (no. 58) the reflex reversal still persists but a distinct after-discharge develops similar to that observed prior to the injection of NaCl but lesser in magnitude. Finally the response returns to normal (no. 59) and it is interesting to note that the reappearance of the reflex inhibition is linked up with the restitution of muscle tone.

This experiment shows that as a result of afferent pain impulses reflex inhibition may be converted into reflex excitation. That the converse is also true is seen in figure 7 in which, under control conditions, only a partial reciprocal innervation seems to exist between the two quadriceps reflexes. No. 12 of figure 7 shows that on stimulation of the left sciatic the right quadriceps reacts with a typical powerful contralateral extensor reflex, but the left quadriceps instead of showing reflex inhibition contracts briefly at the onset of the stimulation and shows a slight rebound and after-discharge. The phase of increasing tension after the cessation of stimulation coincides with the fall in tension of the right quadriceps muscle. After pain fibers have been excited through NaCl injection into the right hamstrings the tone in the right quadriceps falls and its reflex tension and after-discharge are diminished corresponding to the rules described above. The left quadriceps, however, shows an increase in tone and reacts on stimulation, no longer with an initial contraction but with a marked inhibition followed by a slight rebound and after-discharge. The experiment is reversible and at no. 16 the left quadriceps reacts as it did under control conditions. That the alteration in tone and probably the proprioceptive reflexes set up under these conditions are an essential factor in determining the reflex response is suggested by the record of no. 17 (fig. 7) in which by increasing the initial tension in the left quadriceps in the absence of pain reflexes a reflex reversal is obtained.

Discussion. The present experiments confirm and extend earlier work in showing that afferent pain impulses originating in striated muscle profoundly alter excitatory and inhibitory reflex effects. The fact that in experiments on decerebrated animals anesthesia is dispensed with accounts for the great sensitivity of these preparations to minute quantities of hypertonic NaCl solutions (0.1 cc.) and for the prolonged effects of these pain impulses.

Whereas, similar to experiments on the knee jerk reported earlier, the effect of contralateral NaCl injection on the crossed extensor reflex is variable in animals with little extensor rigidity, consistent results are obtained when cats with marked rigidity are used, and when the initial tension under which the extensor reflex is recorded is considerable. Under the latter conditions it is found that contralateral[7] pain impulses increase and ipsilateral pain impulses decrease the

[7] With respect to the recorded muscle.

extensor reflex. These observations suggest that pain impulses have a dual effect on the reflex centers involved in the crossed extensor reflex:

1. An inhibitory action which is observed not only in all experiments involving ipsilateral NaCl injection, but also in about 50 per cent of the experiments in which NaCl is injected into the contralateral side provided that rigidity is slight.

2. A strictly contralateral excitatory effect which, indicated by an increase in tone, follows injection of hypertonic NaCl solution. This excitatory effect is seen not only in the increased initial tone but in the increased reflex effects during stimulation and immediately afterwards (rebound and after-discharge). It is suggested that in preparations with great rigidity and high initial tension in which NaCl injections are accompanied by marked changes in tone, the extensor reflex center becomes more responsive to excitatory stimuli because the maintenance of the extensor tone at a higher level furthers facilitation of the extensor reflex centers through proprioceptive reflexes. Likewise ipsilateral NaCl injection causing a diminution of the facilitatory effect of proprioceptive reflexes on the reflex centers by lessening the extensor tone thereby contributes to their diminished response to sciatic stimulation. Just as voluntary innervation of the extensor muscles exerts a facilitatory influence on proprioceptive reflexes (Hoffmann, 1922) so facilitation exists between proprioceptive and exteroceptive reflex impulses impinging upon the same reflex center. If the rigidity is small and, consequently, only very slight changes in tone result from injection of hypertonic NaCl, no additional proprioceptive reflexes are set up and the pain impulses affect the extensor centers by inhibition only. In line with this interpretation is the fact that in those preparations with low extensor rigidity in which contralateral NaCl injection caused an increase in the extensor reflex a slight increase in tone was noticeable (cf. fig. 3b no. 48) but the muscle tension was unchanged when the contralateral NaCl injection caused a decrease in the reflex response.

This discussion makes it understandable that ipsilateral NaCl injection diminishes the muscle tone of the quadriceps and its reflex response to contralateral stimulation of the sciatic and that, conversely, contralateral NaCl injection increases muscle tone and contralateral extensor reflex. However, the changes of the inhibitory reflex response under the influence of afferent pain impulses have not yet been explained. It was shown that the inhibition of the quadriceps tone on ipsilateral stimulation of the sciatic was related directly to the degree of tone present at the onset of the stimulation. If NaCl was injected into a muscle of the left leg it increased the tone of the right quadriceps and consequently the inhibitory effect of ipsilateral (right) sciatic stimulation was increased. On the other hand NaCl injection into a muscle of the right leg diminished the tone of the right quadriceps and a lesser inhibitory response resulted from ipsilateral (right) sciatic stimulation. It may appear to be paradoxical that the quadriceps in a state of increased tone responds to a standard reflex stimulus with an increased reflex inhibition since the central excitatory and inhibitory state under conditions of interaction are known to "suffer a mutual quantitative inactiva-

tion" (Creed, Denny-Brown, Eccles, Liddell and Sherrington, 1932). Therefore a stimulus producing a given degree of inhibition should become less effective when it is imposed upon nerve centers whose excitatory state is increased. However, the experimental results show that the opposite is the case.

In view of the fact that the inhibitory process in the antagonist reflects quantitatively the excitatory process of the agonist under conditions of reciprocal innervation (Sherrington, 1940) a consideration of the behavior of the agonist is important. The experiments on the contralateral extensor reflex reported above show that the reflex response is directly related to the tone induced by afferent pain impulses. This result was thought to be due to the facilitatory effect of proprioceptive impulses on the reflex excitation on the extensor centers set up by stimulation of the sciatic nerve. It appears highly probable that a similar relation would hold for flexor reflexes of the decerebrate cat and that tone and reflex response are likewise directly related in the case of the flexor reflexes. Rademaker and Hoogerwerf (1929) observed that reflex stimuli which influence the state of decerebrate rigidity act upon the tone of the flexor and extensor muscles in the same manner. Thus, turning the head to the right increases the muscle tone in flexors and extensors of the right side. Afferent impulses set up by hypertonic NaCl injection act in a similar manner.[8] The action potentials of flexors and extensors are increased on the contralateral but decreased on the ipsilateral side. These experimental observations together with our discussion furnish adequate data to explain the alteration in the excitatory and inhibitory processes involved in the contralateral extensor reflex. Apparently the increased inhibition to ipsilateral stimulation of the sciatic which is observed when the extensor tone is increased on account of contralateral NaCl injection is just the reflection of the increased flexor reflex which occurs under these conditions. Likewise, if under the influence of ipsilateral NaCl injection the tone of the quadriceps is decreased and the reflex inhibition in response to ipsilateral sciatic stimulation is lessened, the alteration in the reflex inhibition is only the quantitative reflection of the change of the reflex response of the flexor muscle which must be diminished under these conditions.

The experiments discussed thus far have shown that afferent pain impulses originating in striated muscle have a considerable quantitative effect on excitatory and inhibitory reflex processes. However, certain qualitative changes in the general character of extensor reflexes have not yet been considered. As has been shown above, a reflex reversal may be the result of afferent pain impulses. Although under control conditions the quadriceps reacts to contralateral nerve stimulation with a contraction and to ipsilateral stimulation with an inhibition, this form of reciprocal innervation may be temporarily altered as a result of afferent pain impulses. It has been found that this result occurred particularly when the tone on the "inhibitory side" was greatly lowered. Thus under control conditions in response to ipsilateral nerve stimulation reflex inhibition occurred when the initial tone was high, but reflex excitation appeared in the same muscle after it had lost most of its tone as a result of pain impulses. As the effect of

[8] Unpublished observations.

the afferent pain impulses vanished the tone recovered and the inhibitory response was restored. Apparently the alteration in tone is pre-eminently involved in determining the reflex response. This is borne out by the fact that in preparations in which the muscle tone is low the two quadriceps frequently reacted with a co-contraction to sciatic stimulation instead of showing reciprocal innervation. Moreover, attention has been called earlier to the observation that in preparations in which in response to ipsilateral nerve stimulation a reflex excitation instead of reflex inhibition appears, the latter response may be established by recording the activity of the quadriceps muscle under a greater initial tension. Although details are ill understood at the present time proprioceptive impulses seem to play an important rôle not only in the quantitative changes in excitatory and inhibitory reflex responses set up by afferent pain impulses, but also in the qualitative reflex response which determines whether reciprocal innervation or co-contraction follow ipsilateral nerve stimulation in the decerebrate animal.

A final note should be added concerning the nature of the nerve fibers stimulated in these experiments. Obviously, the injection of a hypertonic solution is apt to stimulate any nerve fibers present in striated muscle. If, in the present as well as in previous investigations of this series, the effect of NaCl injection was related to its action on pain fibers this intrepretation was based on the following data:

1. It was found earlier (Thompson and Gellhorn, 1945) that effects on spinal reflexes paralleled those on pupil and vocalization which accompany pain.
2. Excitation of proprioceptive fibers is ordinarily restricted to the particular muscle whose spindles have been stimulated (Hoffmann) whereas in the present experiments stimulation of nerve fibers in the hamstrings by NaCl had the same effect on the quadriceps reflex as that of other muscles of the same leg.

SUMMARY

In cats with marked decerebrate rigidity it is found that hypertonic NaCl solution when injected into striated muscle sets up afferent pain impulses which greatly modify excitatory and inhibitory reflex processes. Afferent pain impulses increase the tone of the muscles of the opposite side and diminish it on the same side. Under these conditions it is shown that the intensity of the contralateral extensor reflex response is directly related to the tone as determined by the afferent pain impulses. This statement likewise applies to other excitatory phenomena of the extensor reflex such as rebound and after-discharge which also increase with increasing muscle tone. It is furthermore found that the ipsilateral reflex inhibition is altered as a result of afferent muscle pain impulses. The inhibitory response is again directly related to the tone of the muscle which is partly determined by afferent pain impulses. Since it is shown that under the influence of afferent pain impulses flexor and extensor tone are modified in a similar fashion it is assumed that the increased inhibition of the extensor muscle seen under conditions of increased tone is only a reflection of the increased flexor reflex which occurs under these conditions. The fact that in preparations with low rigidity inhibitory effects prevail even on the contralateral side as a re-

sult of afferent pain impulses makes it appear probable that the latter exert a double (excitatory and inhibitory) effect on spinal reflex centers.[9]

It is demonstrated that afferent pain impulses may alter the excitatory and inhibitory reflex response not only quantitatively but also qualitatively; they may lead to a reversal in the reflex pattern. Reciprocal innervation may be converted temporarily into co-contraction as a result of pain impulses and co-contraction may temporarily be changed into reciprocal innervation. These changes seem to be dependent on the alteration in muscle tone set up by afferent pain impulses.

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[9] The fact that dual effects may result from electrical stimulation of mixed nerve fibers has been emphasized by Creed and collaborators (p. 112).

METABOLIC FACTORS IN OXYGEN POISONING[1, 2]

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In a comprehensive review of oxygen poisoning, Stadie, Riggs and Haugaard emphasized that the basic chemical pathology of the syndrome is a function of the reversible or irreversible oxidation of cellular components some of which are related to enzymatic activity (1). It is obvious that the oxidation of prosthetic groups like sulfhydryls would be influenced by the rate of metabolism of the animal as a whole, or of specific tissues, and that the rate would be accelerated or depressed by differences in metabolism. Accelerated oxidation of the reduced compounds should be associated with a shortening of the time of onset of the seizure characteristic of oxygen poisoning, while depressed oxidation should be associated with a longer time of onset. This hypothesis has been tested in the investigation reported here of metabolic factors involved in oxygen poisoning, and has been found to be true. The data are presented under several headings, each of which includes a brief summary of the most pertinent details of the literature, technical details of procedure, results, and discussion of their possible significance. The subjects fall under the following classification: 1. Effects of hypo- and hyperthyroidism. 2. Effect of environmental and body temperature. 3. Changes in body temperature during oxygen poisoning. 4. Relative susceptibility of young and adult cats. 5. Effect of drugs.

Effects of hypo- and hyperthyroidism. The only studies on this subject are those reported by Campbell (2). He reported that thyroxin enhanced the effects of oxygen poisoning in rats while thyroidectomy and to a lesser extent, hypophysectomy (3) protected the animals from the effects of similar exposure to oxygen. These conclusions were based on the survival time after exposure to high oxygen pressures. Since the convulsive seizure and gross neurologic effects of oxygen poisoning may be only partially displayed in rats, the conclusion had to be based on the survival time, a much more complex factor which might be influenced by pulmonary, renal or pancreatic defects as well as by neurologic defects. It was of some interest to separate these factors by observations on nine cats. The time of onset of the oxygen seizure of two cats (one thyroidectomized, the second treated with thyroid extract) was obtained after daily exposure of the animals to oxygen at 105 lb. per sq. in. (gauge). The animals

[1] This article has been released for publication by the Division of Publications of the Bureau of Medicine and Surgery of the U. S. Navy. The opinions and views set forth in this article are those of the writer and are not to be considered as reflecting the policies of the Navy Department.

[2] The experiments reported here constitute one aspect of a determined effort by members of the Institute to add to our understanding of the problems involved with the purpose in mind of removing dangers to diving personnel and of increasing their efficiency.

were decompressed in one minute and removed to air, and notes made on the severity of the seizure. Basal metabolic measurements were also made. When the animals were killed, any tissue from the neck suspected of containing thyroid tissues was sectioned serially. It is unfortunate that only one cat was successfully thyroidectomized, several attempts to remove the glands in other animals having failed. Two other hyperthyroid cats were tested under more favorable conditions, and the results are described in a later paragraph.

The time of onset of the oxygen seizure was increased in the thyroidectomized cat (fig. 1). The cat frequently remained at levels which would have been lethal or severely damaging to normal cats. In addition, the convulsive seizures were almost invariably very mild and of short duration, the animal showing no neurological defects at the conclusion of the experiment. There was no weight loss during the experiment. The basal metabolic rate, measured by the open

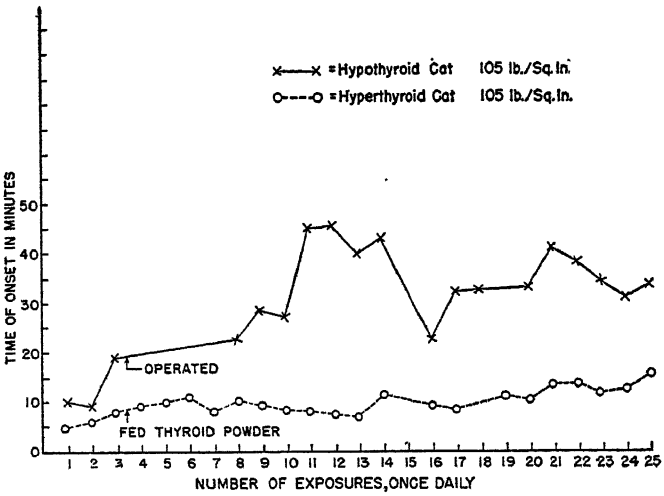

Fig. 1. Time of onset of convulsions of hypothyroid and hyperthyroid cats

chamber method, was 213 cc. carbon dioxide output per kgm. per hour, as compared with 318 to 485 cc. for five normal cats run at the same time. Microscopic examination of the neck fascia revealed several parathyroid glands and a small amount of thyroid tissue consisting of small follicles with low cuboidal epithelium inclosing little or no stainable colloid.

The hyperthyroid cat (fed 0.5–1.0 gram thyroid powder (U.S.P.) per day) was the first animal of a large series studied (4) which showed a reduced tolerance, even if slight, to oxygen poisoning on repeated exposure (fig. 1). The lowest level was reached on the tenth day of thyroid feeding, which is what one would expect of a true thyroid action. The subsequent rise in time of onset is also to be expected as typical of the action of thyroid extract. The seizures were very prolonged (lasting frequently for ten minutes) and severe. Furthermore, the condition of the animal at the close of the experiment was very poor. It was partially blind and showed other neurologic defects. The basal metabolic rate

ten days after the inception of thyroid treatment expressed as 685 cc. production of CO_2 per kgm. per hour, was about one and one-half times greater than the highest normal value.

These observations on the effects of hyperthyroidism were confirmed on two additional cats. In table 1 the time of onset of the seizure is given for the animals before the administration of thyroid powder, after ten days' treatment with dried thyroid, and 12 days after withdrawal of the drug, at a time when the metabolism would be expected to return to normal. Each animal was given an oxygen seizure on each of two or three successive days in each condition. To prevent excessive damage, cats were not exposed to oxygen under pressure for more than sixty minutes.

It would seem that hypothyroidism may reduce the time of onset of the convulsive seizure, the severity, and the cumulative neurologic damage caused by the effect of oxygen under pressure on the central nervous system, while on the other hand, hyperthyroidism increases sensitivity.

Effect of environmental and body temperature. The effect of variations in the environmental temperature on oxygen poisoning was studied by Campbell (5).

TABLE 1

Effect of hyperthyroidism on the time of onset of the oxygen seizure

CAT NO.	PRESSURE LB. SQ. IN.	TIME OF ONSET OF MOTOR SEIZURE IN MINUTES		
		Normal control	Hyperthyroid, after treatment with thyroid	Presumably normal after withdrawal of thyroid powder
3	60	60, >60, >60	13, 15, 12	>40, >50
4	60	>60, >60, >60	11, 13, 19	>30, >60

He found that when rats are subjected to six atmospheres of oxygen for 30 to 60 minutes, the number which survive is far less when the environmental temperature is at 33°C. than at 24°C. He believed that oxygen poisoning was much enhanced by preventing the fall in body temperature which he found to accompany the syndrome, by raising the environmental temperature to the higher value. There was some question about carrying over Campbell's conclusions to the neurologic aspects of the syndrome in cats for the following reasons: 1. The unit of measurement applies to the animal as a whole, without any necessary relationship to the neurologic effects. 2. Rats may not manifest regularly early altered activity of the central nervous system characteristic of man or the cat. 3. Changes of environmental temperature are not necessarily reflected in correspondingly large fluctuations in body temperature in furred animals. To learn whether the nervous system was influenced by temperature in the same way, experiments were repeated on cats, the time of onset of the convulsive seizure being taken as a measure of neurologic damage.

In the first experiment, cats were exposed to oxygen at 105 lb. per sq. in. at room temperature (22°C.) The procedure was repeated on each of four successive days, the environmental tank temperature being 4°C., 22°C., 30°C. and

41°C. The seizure time was noted in each case. Table 2 is a summary of the average seizure times.

It is clear that changes in environmental temperature as large as 20°C. do not affect significantly the seizure time of cats subjected to high pressure atmospheres of oxygen.

In the second experiment, cats were depilated and control seizure times were obtained before and after cooling or warming by immersion in water. Rectal temperatures preceding and following the seizure were noted, together with the

TABLE 2

Time of onset of seizure of cats exposed to oxygen under pressure at different environmental temperatures

	TEMPERATURES °C.	NUMBER OF CATS	AVERAGE SEIZURE TIME
			minutes
First control	22	9	13.3
	4	10	13.6
	30	9	14.7
	41	10	14.3
Last control	22	10	14.7

TABLE 3

Effects of changes in body (rectal) temperature on the time of onset of the oxygen convulsion

CONTROL TIME	NORMAL RECTAL TEMPERATURE	RECTAL TEMPERATURE PRECEDING O_2 EXPOSURE	SEIZURE TIME
minutes	*°F.*	*°F.*	*minutes*
52	102.6	92.6	25
42	102.6	92.0	21
19	102.6	92.6	13
26	101.2	93.8	21
>60	101.2	105.4	29
28	103.2	106.0	22
59	103.8	102.0*	23

* Rectal temperature after exposure to warm water.

seizure time. The cats were subjected to an atmosphere of oxygen at 60 lb./sq. in. (gauge), decompressed at the first signs of convulsive activity and removed to air. The results (table 3) show that either decrease or increase of body temperature results in an increased susceptibility of cats to neurologic damage as manifested by the onset of a motor seizure.

It is difficult to compare Campbell's experiments with those reported above, for the criteria of the toxicity of oxygen as well as the animals used were different. In addition, the central nervous system of the cat seems to be more sensitive to the effects of oxygen than that of the rat. In the experiments on cold, it

should be noted that all cats shivered constantly, and this must have been accompanied by a rise in metabolism. The decrease of the seizure time might be expected to follow the rise in metabolism.

Changes in body temperature during oxygen poisoning. Paul Bert (6) was the first to call attention to the marked drop in body temperature which takes place in animals suffering from oxygen poisoning. This has since been confirmed by other workers. Bean, however, found no change occurred in rectal temperature (7). Hederer and André (8) found in rabbits that the body temperature rises slightly during the convulsion and then falls below normal. It seemed unlikely that lowering of the body temperature by as much as 10°C. could take place in

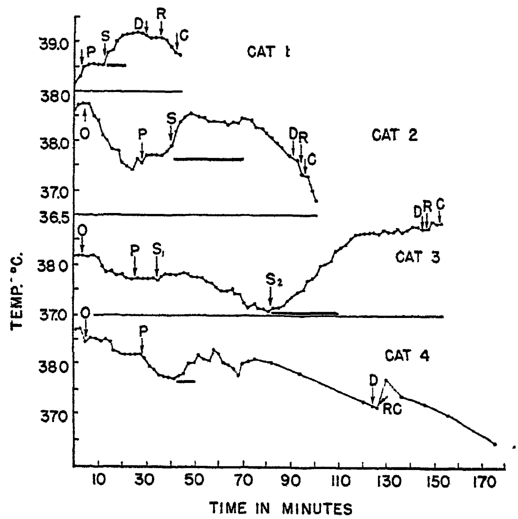

Fig. 2. Changes in rectal temperature of cats exposed to oxygen at a pressure of 105 lbs. per square inch. *O* = oxygen at atmospheric pressure; *P* = pressure applied; *S* = first seizure; *D* = decompressed; *R* = respiration ceased; *C* = heart ceased beating; — = duration of seizure.

cats which had convulsions early after exposure to oxygen at high pressures. The experiment was therefore repeated. The cat was securely confined in a tray, a thermocouple inserted about four inches into the rectum, and the temperature was recorded every minute in most experiments in air, in oxygen, under pressure at 105 or 75 lb./sq. in. (gauge), during decompression, and after the animal was returned to air. Notes were made of the activity of the animal. The results (fig. 2) may be summarized as follows: 1. The administration of oxygen at atmospheric pressure was accompanied by a fall in body temperature. This may have been due to lessened struggling. 2. On the application of pressure the body temperature levelled off in three cases and continued to fall in a fourth. 3. The seizure was accompanied by a slight rise in rectal temperature in all cases. This might be maintained up to the end of motor activity and through the decompression phase to the death of the animal. 4. Visible motor activity,

no matter how slight was almost always accompanied by an appreciable rise in rectal temperature. 5. The changes in rectal temperature were never great, and did not exceed a maximum of two degrees centigrade. It is difficult to explain the enormous fall in body temperature described by Bert and others, except on the basis of a moribund or nearly moribund state.

These results were confirmed by a second group of experiments in which cats were also depilated. The pressures used were lower (75 or 60 lb./sq. in.) in order to prolong the pre-convulsive phase to accentuate possible changes in body temperature. Rectal temperatures were recorded before the animal was subjected to oxygen under pressure and after it had recovered from the seizure. The cats were decompressed to atmospheric pressure during one minute after the inception of the seizure, and the seizure was completed in air at atmospheric pressure. Ten convulsions were accompanied by a rise in body (rectal) temperature and an equal number resulted in a fall, while, in one instance, no change took place. The mean rise in rectal temperature was +0.80°C., the mean fall −0.88°C. (table 4).

TABLE 4

The effect of oxygen seizure on rectal temperature

CAT NO.	6	7	8	9	10
Normal rectal temp. minus temp. after seizures	+1.0 +0.5 0	−0.9 −1.0 −1.5 −1.4	+0.6 +0.6 +0.6	−0.6 +1.2 −0.3 −0.3 −0.1	+1.2 +0.4 +1.6 −2.5

Relative susceptibility of young and adult cats. Smith and his co-workers (9, 10) showed that young rats have a greater survival rate, and show less pulmonary injury than older rats after exposure to less than one atmosphere of oxygen. The same relative insensitivity to the effects of oxygen under high pressure apparently exists also in kittens, at least so far as neurologic damage is concerned, as the following experiment indicates. Five kittens were exposed daily to oxygen at a pressure of 105 lb./sq. in. (gauge) until the first signs of convulsions appeared. They were then decompressed, and returned to the mother. Exposure to oxygen was begun one to seven days after birth. The record of the time of onset of the seizures given in figure 3 shows that as compared with the normal, there are several striking differences: 1. The time before the seizure is greater. 2. It decreases as the number of exposures progresses, as compared with the invariable increase in otherwise normal adults. 3. Only after nearly a month does the seizure time approach what it might have been in an adult cat exposed to oxygen in a similar manner. The exposed kittens gained less in weight than control litter mates, were less playful, and were still nursing the mother at the end of the experiment, although the control kittens were weaned. In addition to being retarded, the exposed kittens were ataxic at the end of the experiment.

The greater resistance of young kittens to oxygen poisoning applies thus not only to pulmonary structures but also to the central nervous system. It may be that the relative insensitivity of the central nervous system to oxygen under high pressures resembles the resistance to the effects of arrest of the cerebral circulation in very young animals, and to other forms of anoxia (11, 12). It has been suggested that survival of relatively long periods of anoxia by the brain of newborns is due to the ability of the neurons to obtain energy anaerobically, and that this function is gradually reduced during growth. The same argument may be applied to explain the greater resistance of immature kittens to the effects of oxygen poisoning. It is possible that essential reducing substances present in nerve tissue are oxidized in immature as in mature cortices, but that an accessory energy-producing system is present in the immature tissues which can continue to function until it, too, is interfered with. Proof

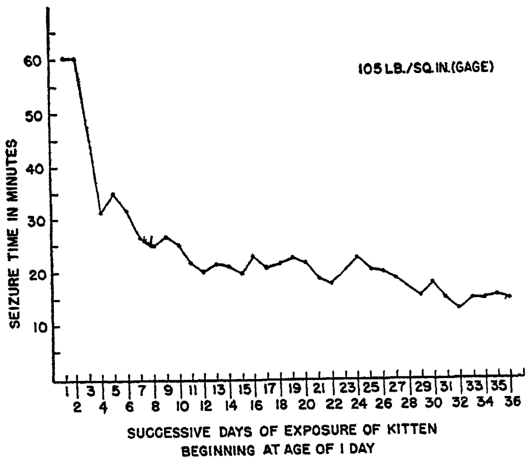

Fig. 3. Time of onset of convulsion of kitten beginning at age of one day

that several successive (respiratory) enzyme systems appear during development of the central nervous system has been supplied by Flexner et al. (13).

Effect of drugs. Two types of drug action affecting metabolism may be noted: 1. Depression or stimulation of the metabolism of the central nervous system. Depressive agents (amytal and pentobarbital sodium in doses as low as 5 mgm. per kgm., urethane, chloral hydrate and paraldehyde) all delayed or prevented the onset of the convulsive seizure induced by oxygen under pressure. Stimulating agents (metrazol and, to a lesser degree, acetylcholine) hastened the time of onset of the convulsion. 2. Depression of the metabolism of the animal as a whole. Thiourea given prophylactically in large doses over a period of weeks was without effect on the time of onset. Examination of the thyroid gland did not show the cellular reaction which occurs when thiourea reduces metabolism in the rat, and it is doubtful that there was in the cat an actual reduction in metabolism. Another metabolic depressant acting through the

thyroid is 2-thiouracil, which was administered in the same way. It may have had a slight effect in delaying the onset of the convulsion. Microscopic examination of the thyroid glands of these animals does not indicate that this drug has any marked thyro-inhibitor activity.

SUMMARY AND CONCLUSIONS

1. Hypothyroidism lengthens the time of onset of the convulsive seizure and decreases the cumulative neurologic damage. On the other hand hyperthyroidism increases the sensitivity of the animal.

2. Increase or decrease of the environmental temperature by about 20°C. has no effect on the motor seizure. Either increase or decrease of the body (rectal) temperature reduces the time of onset of the seizure.

3. Kittens are less sensitive to oxygen poisoning than adult cats.

4. The rectal temperature is raised slightly during the motor seizure. The body temperature falls only as the animal becomes moribund.

5. Drugs which depress the metabolism of cortical activity delay the onset of the convulsion. A convulsant drug (metrazol) accelerates it.

6. The results point to a profound effect of changes in metabolism on the convulsive seizure of oxygen poisoning.

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THE EFFECT OF ELECTRICAL STIMULATION ON NEUROMUSCULAR REGENERATION[1]

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The results of most investigations indicate that it is possible by means of electrical stimulation to retard the atrophy which occurs in skeletal muscle following denervation. The retardation of atrophy is quite consistent and appreciable if the treatments are carried out under conditions which are favorable for the development of maximum tension by the muscle fibers. The preservation of any appreciable mass of contractile tissues by this means would appear a priori to offer advantages to the muscle regeneration which follows reinnervation. The advantages might result from a lessening of fibrosis, a condition which is considered to be an impediment to reinnervation, and from the existence of a larger and stronger muscle fiber at the onset of functional reinnervation. The object of this investigation was to determine the over-all effects upon neuromuscular regeneration of a regimen of electrical stimulation which had been proven to be effective in delaying denervation atrophy and to determine the time after denervation during which electrical treatments are most effective. The experiments were carried out on the gastrocnemius muscles and tibial nerves of adult albino rats. The selection of the rat as an experimental animal was made because considerable information concerning atrophy and regeneration was already available for this species (1) and because it was necessary to employ a large number of animals to permit statistical analysis of the results.

Experimental. A number of control experiments were carried out to permit a better evaluation of the data concerning the effects of electrical stimulation upon neuromuscular regeneration. In 35 unoperated normal animals, comparisons were made of the weight and strength of the right and left gastrocnemii and the capacity of the tibial nerves to elicit tension in their respective muscles. Analyses of the data from these experiments indicate what degree of variability is to be expected in the measurements of muscle weight and strength in the two limbs. An investigation was made concerning the effects of section of one sciatic nerve upon the weight and strength of the contralateral gastrocnemius and the capacity of its tibial nerve to elicit tension in the muscle. This was deemed necessary in view of the question raised by Weiss (2) as to the validity of using data from the muscle and nerve of the unoperated limb as controls for the corresponding tissues of a denervated limb. For this purpose 88 rats, at approximately 90 days of age, were divided equally by lot into two groups. In the experimental group a section of one sciatic nerve was removed. The strength and weight of the contralateral gastrocnemius and the response of the muscle to tibial nerve stimulation in the members of the operated group were compared with those in

[1] Aided by a grant from The National Foundation for Infantile Paralysis, Inc.

the unoperated control group at the end of either two or six weeks. Inasmuch as atrophy and regeneration are concomitant states for a time during the course of neuromuscular regeneration, it was advisable to determine the effects of daily electrical stimulation upon normal innervated muscle fibers.

Experiments to determine the effects of electrical stimulation upon muscular atrophy and neuromuscular regeneration were carried out on a total of 120 animals. The general plan was to denervate the gastrocnemius muscles of both limbs by crushing their tibial nerves. One limb was subjected to daily treatments with electrical stimuli and the non-stimulated contralateral limb was employed as a control. At designated times after denervation, determinations were made as to the weight and strength of the treated and untreated muscles and the capacity of the regenerating nerves to activate their muscles. The techniques which were employed for nerve crushing, stimulation and strength measurements have been described elsewhere (1). The experimental muscles were given daily treatments with strong faradic shocks for a period of 22 seconds. The type of treatment employed was that previously found to be effective for the retardation of atrophy in the totally denervated gastrocnemius of the rat (3). Such treatments caused no significant increase in the tension of the contralateral control gastrocnemius muscle. Observations were made as to the time after denervation at which the earliest signs of motor reinnervations appeared in the treated muscles and their untreated contralateral controls. This was done by the application of volleys of slightly supermaximal condenser discharge stimuli to the regenerating nerve and observing with the aid of sensitive isotonic levers the presence or absence of muscle contractions. Determinations of the strength and weight of the treated and untreated muscles were made at either 7, 12, 14, 21, 24, 28 or 35 days after denervation. On some animals the electrical treatments were instituted the day following denervation and given daily until the termination of the experiment. In other experiments the treatments were discontinued some days prior to the time of the strength and weight determinations. The experimental procedures employed made it possible to evaluate separately the effects of electrical stimulation upon the rate of denervation atrophy, the time required for the earliest signs of functional reinnervation to appear and the rate and extent of muscle regeneration subsequent to reinnervation. It was also possible to determine the extent to which the differences between treated and untreated muscles are equalized in the later phases of regeneration. These experiments permit an evaluation of the end results of a regimen of daily electrical stimulation initiated immediately following denervation and continued until a considerable degree of regeneration had occurred.

Results. The data (table 1) show that no significant differences for weight and strength were found between left and right gastrocnemii of normal non-operated animals. It was observed (table 2) that the section of one sciatic nerve had no effect upon either the weight and strength of the contralateral gastrocnemius muscle or the capacity of the contralateral tibial nerve to activate its muscle. These findings indicate that, if unilateral nerve section caused morphologic changes in contralateral intact nerves comparable to those described by Tamaki

(4) and Greenman (5), they were unaccompanied by changes in the weight and strength of the gastrocnemius muscle or the capacity of the nerve to elicit tension in its muscle. Such evidence supports the validity of employing contralateral controls in unilateral denervation studies.

TABLE 1

Average values together with standard errors for right and left gastrocnemii of normal unoperated rats and for the effects of daily electrical stimulation upon normal muscle

EXPERIMENTAL CONDITION	NO. OF ANIMALS	TIME OF TREATMENT	MUSCLE WEIGHT	ISOMETRIC TENSION PER		TENSION (NERVE*) / TENSION (MUSCLE*) × 100
				Muscle	Gm. muscle	
		days	gms.	gms.	gms.	
Left	35	0	1.243 ±0.049	2050 ±80	1648 ±71	90.1
Right	35	0	1.240 ±0.047	2049 ±80	1658 ±78	89.8
Stimulated	15	14	1.468 ±0.070	2192 ±106		
Non-stimulated	15	14	1.463 ±0.063	2373 ±108		
Stimulated	10	28	0.939 ±0.032	1563 ±69		
Non-stimulated	10	28	0.912 ±0.034	1529 ±56		

* Stimulated via.

TABLE 2

Effects of nerve section upon muscle and nerve of unoperated contralateral limb

NO. OF ANIMALS	DAYS AFTER OPERATION	MUSCLE WEIGHT / BODY WEIGHT × 100	GMS. MUSCLE TENSION / GMS. BODY WEIGHT	TENSION (NERVE*) / TENSION (MUSCLE*) × 100
25	controls	0.663	10.58	88.9
23	14	0.666	10.70	89.3
19	controls	0.643	10.83	90.4
20	38	0.655	11.29	91.5
44	all controls	0.654	10.69	89.5
SEM		±0.006	±0.17	±1.2
43	all exper.	0.661	10.97	90.4
SEM		±0.007	±0.21	±1.1

* Stimulation via.

The earliest signs of functional motor reinnervation in the gastrocnemius muscle following a lesion made by crushing the tibial nerve at the junction with the peroneal nerve occur 12 to 15 days after operation in the adult albino rat. Soon thereafter atrophy is arrested and regeneration takes place at a uniform

and predictable rate (1). In our experiments no consistent differences were observed between the stimulated and non-stimulated limbs as to the time required for the onset of functional reinnervation. This finding indicates that electrical stimulation neither hastens nor retards the progress of axone outgrowth and its establishment of functional contacts with denervated muscle.

In the animals studied at 7, 12 or 14 days following denervation, the stimulated muscles were found to be consistently heavier and stronger than their untreated contralateral controls (fig. 1). The muscles can be considered as being

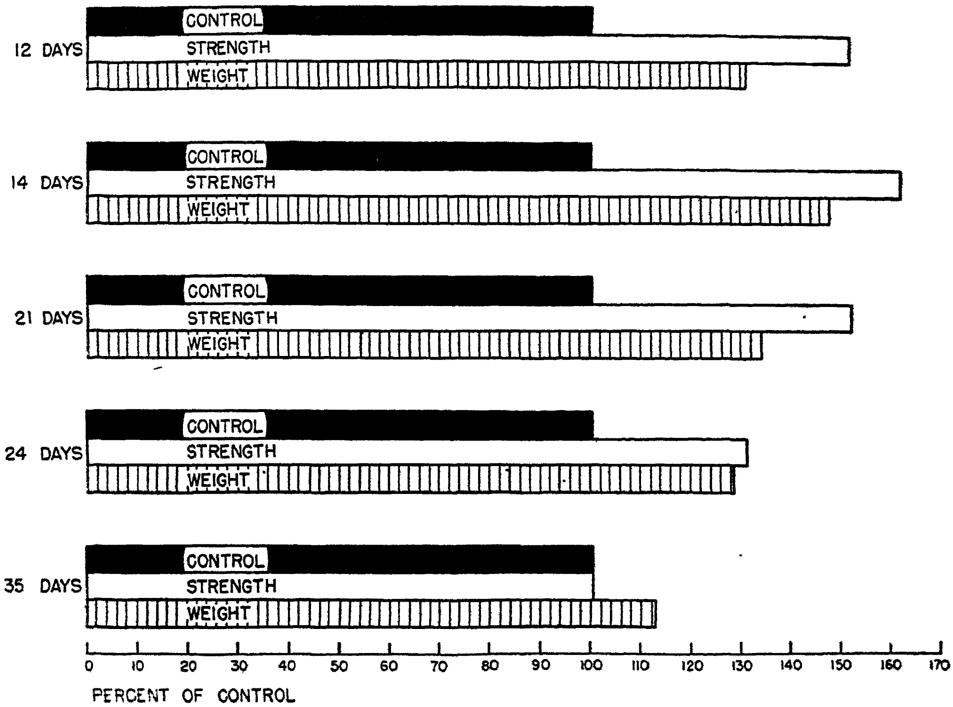

Fig. 1. A graph showing average values for the effects of daily electrical stimulation upon neuromuscular regeneration. The black bars represent average values on non-stimulated devervated contralateral control muscles expressed as 100 per cent. The time in days designates the duration of treatments and time after denervation at which measurements were made.

essentially without innervation during these periods of time. Thus electrical stimulation by retarding muscle atrophy enables the regenerating axones to make functional contacts with larger and stronger muscle fibers. Such treated muscles have been found to have higher creatine and glycogen concentrations than untreated denervated control muscles but possess comparable water concentrations. The greater strength of the stimulated muscles is attributed to their larger mass. Treatments with electrical stimuli failed to prevent the loss of strength per unit mass of muscle which occurs following denervation. This finding is in agreement with the observations of Fischer (6) and Eccles (7) that electrical

stimulation fails to arrest the gradual loss of contractile strength in denervated muscle.

The experiments on animals which had received daily treatments with electrical stimuli for 21, 24 or 35 days after denervation show that the differences between treated and untreated muscles were gradually equalized in the days following reinnervation (fig. 1). The equalization of weight and strength differences appeared to be a gradual process. At 35 days after denervation there was no difference between the strength of the treated and untreated muscles and only a small difference between the muscle weights. The experiments in which the electrical treatments were delayed until after the onset of initial reinnervation showed comparatively little beneficial effects from electrical stimulation.

The data (table 1) show that the daily treatment of normal muscles with the same stimulus modality which was employed in the experiments on denervated and regenerating muscles caused no appreciable change in the strength and weight of innervated muscles. The absence of any effects of electrical stimulation upon the mass and strength of innervated muscle indicates that the equalization of differences which were noted to occur after the onset of reinnervation cannot be attributed to injury of nerve and muscle from the continued treatments.

It was possible by means of adequate electrical stimulation to retard the rate of atrophy but not to prevent the gradual loss of contractile strength in denervated muscle. This means that the muscle fibers were larger and, because of this, stronger at the time of initial reinnervation than they would have been without electrical stimulation. This initial advantage was gradually lost despite a continuation of the treatments during the later stages of regeneration. After a time little or no difference was found to exist between treated and untreated muscles. It can be calculated that following reinnervation the untreated muscles actually recovered mass and strength at a faster rate than contralateral treated muscles. Our experiments indicate that any beneficial effects from electrical stimulation result from changes which take place prior to and not subsequent to reinnervation. This suggests the futility of continuing the electrical treatments after the onset of functional reinnervation. However, we noted no deleterious effects of the regimen of treatment upon any phase of neuromuscular regeneration. The rate of growth in regenerating axones and their success in establishing functional contacts with muscle fibers were unaffected by the electrical treatments. Only speculation can be advanced as to whether the over-all effects of electrical therapy would be more beneficial in cases where longer times must necessarily elapse between denervation and reinnervation and where greater degrees of fibrosis are encountered. Such findings might be anticipated from the studies of Gutmann and Guttmann (8) concerning the effect of galvanic stimulation on denervated muscle in which greater differences were found between treated and untreated muscles in cases of late reinnervation. It is apparent that the valuation of the therapeutic effects of electrical treatments in peripheral nerve injuries will be prejudiced by the time after denervation at which critical measurements are made.

SUMMARY

A study has been made of the effects of daily faradic stimulation upon neuromuscular regeneration in the adult albino rat. The experiments were carried out upon the gastrocnemius muscles at various times after crushing the tibial nerves and included measurements of muscle mass and strength and the capacity of the regenerating nerve to activate its muscle.

Daily treatments with electrical stimuli greatly retarded the loss of weight and strength in muscles prior to their reinnervation. The treatments employed appeared to have no effect upon the time of onset of functional reinnervation and upon normal innervated muscle. The electrical stimulation and the stretching and fatigue resulting therefrom appeared to be without injurious effects upon any phase of neuromuscular regeneration. However, when the treatments were continued for some time after reinnervation the differences between the treated nerves and muscles and their untreated contralateral controls were found to be gradually equalized. Thus an evaluation of the effects of electrical treatment will be prejudiced by the time after denervation at which critical measurements are made.

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LIFE CYCLE OF WHITE BLOOD CELLS

The Rate of Disappearance of Leukocytes from the Peripheral blood of Leukopenic Cats[1]

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Our purpose in reporting the experiments recorded in this paper is to show that the life cycle of the white blood cell is not greater than 16 hours in irradiated leukopenic cats. This short life span is probably true within limits for normal cats, also. This is what might naturally be expected in view of the large amount of myeloid and lymphoid tissue in the body, since it would not seem likely that such large potential manufacturing centers would be present unless they were being put to active use.

The life of the white blood cells has been estimated by various investigators with widely varying results. Weiskotten (20), on the basis of results in animals treated with benzol, estimated that three or four days represented the life span of the polymorphonuclear neutrophile. Bunting (3) felt that little could be said with certainty with regard to the life of the lymphocyte. Minot and Isaacs (16) found that transfused leukemic lymphocytes disappeared within two and one-half hours in a human recipient. Tissue culture experiments have demonstrated clearly that white blood cells can be kept alive much longer periods of time. For example, Jolly (8) found that frog's leukocytes could be kept alive over a year. Forti (6) reported motile dog's leukocytes in tissue culture on the seventeenth day, and Margaret R. Lewis (15) demonstrated viable human leukocytes in tissue culture after twenty to twenty-eight days. Ponder (18) came to the conclusion, from study of the nuclear configuration changes in polymorphonuclear neutrophiles of rabbits after injection of thyroid extract, that these cells remained in the circulation of the rabbit for two to three weeks. Ponder, Saslow and Schweizer (19) obtained similar results. Garrey and Bryan (7) called attention to the fact that the blood of new born babies does not contain neutrophiles with polylobulation for nine to fourteen days.

In view of these divergent results we have attempted to arrive at some conclusions as to the time that white blood cells actually remain in the circulating blood.

Method. Normal adult cats were given 1265 to 1965 roentgen units (with the viscera protected by lead shields) to each of the following regions: 1, to head, forelegs and cervical vertebrae; 2, to tail, hindlegs and pelvic girdle; and 3, to upper sacral vertebrae and lumbar vertebrae. Marked leukopenia resulted

[1] The expenses of this work were defrayed in part by a grant from the John and Mary R. Markle Foundation.

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in these animals in three to five days. Leukopenia was produced, also, in one animal by means of infection with the virus of infectious feline agranulocytosis (10, 11).

When the leukopenia was pronounced, cross circulation with a normal cat was established by means of an end to end anastomosis of carotid artery to carotid artery. Frequent white blood cell and differential counts were made on blood obtained by needle puncture of the ears of these animals. After a short period of time (1 to 2 hr.), comparable figures were obtained from each animal of the combination. At varying times after good mixing of the blood of the two connected animals, the anastomosis was discontinued and the animals were returned to their own circulation. Following this the white blood cell picture of the formerly leukopenic animal was followed closely until the number of white blood cells per cu. mm. had returned to the same low level which was present prior to the establishment of cross circulation. The blood picture in the normal animal was followed in the same way during this interval. All determinations of the white blood cells were made with Bureau of Standards pipettes and hemocytometers.

Eight experiments were done using cross circulation between irradiated and normal cats and one additional experiment was performed in which a cat with leukopenia due to infectious feline agranulocytosis was used in place of an irradiated animal.

In one of the experiments in which irradiated cats were used, cross circulation was performed with three normal cats in succession. In this experiment the irradiated animal was connected by carotid artery anastomosis with the first normal cat for four hours. Immediately after this, cross circulation was established with a second normal cat and maintained for eight hours. Finally, cross circulation was established with a third normal cat and maintained for another ten hour period. Thus, cross circulation was maintained in this irradiated leukopenic animal for a total of twenty-two hours. The duration of cross circulation in the other animals varied from two to twenty-one hours, the average being 7.4 hours.

In order to establish the effect of cross circulation on the white blood cell picture of normal animals, six experiments were performed in which a normal cat was connected by means of carotid artery anastomosis with another normal cat. Frequent white blood cell counts and differential counts were made on these animals during the period of cross circulation. One other experiment was performed in which a cat with neutrophilic leukocytosis and a normal cat were used in combination.

Results. Cross circulation between normal animals produced a neutrophilic leukocytosis with a distinct shift to the left in both animals. Reference to table 1 shows that the average white blood cell count of eleven of the twelve normal cats used in these six experiments was 16982 per cu. mm. prior to cross circulation. This figure rose to 29336 per cu. mm. at the end of the period of cross circulation. Thus, there was an average increase of 72.7 per cent for the total white blood cells during the time of cross circulation. This increase was due

mainly to neutrophilic cells as there was a corresponding increase of 132.3 per cent in the stabs and segmented neutrophiles during this period, whereas the lymphocytes and eosinophils showed an average diminution of 50.3 per cent and 71.1 per cent respectively. Even at the beginning of cross circulation, changes of the same nature were present, although they were not so marked. Thus, the total number of white blood cells was increased 11.1 per cent and the number of neutrophiles and stabs was elevated 26.9 per cent, whereas the lymphocytes were diminished 22.1 per cent and the eosinophiles 12.7 per cent at this time. There were no significant changes in the monocytes, few of which were present at any time.

TABLE 1

Synopsis of important data in cross circulation experiments (control group)

CAT NUMBER	TIME OF *X IN HOURS	W.B.C. PER C.MM.			STABS AND SEGMENTED NEUTROPHILES PER C.MM.			LYMPHOCYTES PER C.MM.			EOSINOPHILES PER C.MM.		
		Prior to X	Start of X	End of X	Prior to X	Start of X	End of X	Prior to X	Start of X	End of X	Prior to X	Start of X	End of X
3	12	33100	26100	38400	20191	21663	34560	9268	2088	2304	2648	1827	0
4	12	27700	26200	40300	24366	21746	39191	2216	2620	403	831	786	403
9	4½	15900	12600	24600	12561	10458	21894	2385	1260	1722	318	882	0
10	4½	9200	16900	30100	5704	13773	25284	3036	2619	3612	460	507	301
13	7	7900	13700	24700	3318	10001	22724	3713	2877	1482	869	822	0
14	7	9500	25500	29600	5035	17850	27824	3800	6375	1184	665	1020	296
5	2	23500	19100	27200	17155	12988	22848	4935	5348	4352	940	764	0
6	2	16700	17400	24000	12859	14268	20640	2839	2610	3360	668	522	0
2	4½	no counts done											
15	4½	7600	13100	25600	4332	8515	22272	2888	3799	2816	380	786	256
11	5½	21200	20200	28800	8056	15554	27072	11236	3636	1152	1908	1010	576
12	5½	14500	16800	29400	11455	11928	26166	2320	4704	1764	725	168	1176
Total..		186800	207600	322700	125032	158744	290475	48636	37936	24151	10412	9094	3008
Av. per cat.		16982	18873	29336	11366	14431	26409	4421	3449	2196	947	827	273
Av. % of change.. . . .			*+11.1*	*+72.7*		*+26.9*	*+132.3*		*−22.1*	*−50.4*		*−12.7*	*−71.1*

* Cross circulation.

The principal results obtained in the leukopenic animals are shown in tables 2 and 3. Blood was obtained from the ear of each animal just above the anastomosis. Hence, the blood obtained from any one animal of a pair during cross circulation was predominantly that of the other member of the pair. Reference to table 2 shows that in all instances prior to good mixing the lower counts were found in the blood of the ears of the normal animals, as would be expected under these conditions. There was a prompt diminution in the total number of white blood cells in the peripheral blood immediately after cross circulation was begun. The average number of white blood cells per cu.mm. for the sixteen animals used in eight experiments prior to cross circulation was 9628 as compared with 5147 at the time the first determinations were made after the anastomoses were

TABLE 2

Synopsis of important data in cross circulation experiments (experimental group)

CAT NUMBER	TYPE OF ANIMAL	TIME OF *X IN HOURS	INDEPENDENT CIRCULATION IN HOURS	WHITE BLOOD CELLS PER C.MM.					W.B.C. LOST PER C.MM. PER HOUR
				Prior to X	Start of X	End of X	First after X	End of experiment	
7	Irradiated	21	8	300	9100	6650	3800	250	538
16	Normal	21	9	15500	9200	2650	4400	6000	
1	Irradiated	2½	21	250	15600	15300	11800	100	618
18	Normal	2½	21	24000	3200	12300	21400	47300	
19	Irradiated	3	7½	350	2400	4700	5800	50	973
20	Normal	3	7½	13000	550	3950	9300	23500	
23	Irradiated	4	6	600	3700	6300	4050	800	591
24	Normal	4	6	14000	900	5500	7500	14100	
25	Irradiated	2	9	250	8500	9400	10000	100	1213
26	Normal	2	9	19500	1200	9500	14400	19800	
28	Irradiated	6	8	50	6800	7800	6400	550	780
27	Normal	6	8	29700	2300	7800	18700	11100	
29	Irradiated	16	2½	350	4700	2400	1100	250	378
30	Normal	16	9½	13800	2100	1400	2800	20800	
17	†Ag.	5	7	2900	9000	7100	9800	1600	1364
8	Normal	5	7	19500	3100	9000	9900	12100	
Total ..				154050	82350	111750	141150	158400	
Av. per cat				9628	5147	6984	8821	9900	
Av. % of change					*−46.5*	*−27.5*			
Total of 8 normals				149000			88400	154700	
Av. per normal cat							11050	19337	
Av. % of change (normal cats)								*+75*	
31	Irradiated	22	9	1600			12800	1000	1475
21	Normal	4		14300				15300	
32	Normal	8		15600				18000	
22	Normal	10		14000				23800	
Av. per W.B.C. c.mm. per hour									881

* Cross circulation.

† This cat had infectious feline agranulocytosis.

established. Thus, there was a percentage drop of 46.5. This reduction in the total number of the white blood cells became less as cross circulation was continued but was still 27.5 per cent shortly before the animals were disconnected. There was no close correlation between the degree of drop in the white blood

cells and the duration of the cross circulation (except during the first hour of cross circulation when low values were constantly found), although two of the lowest figures—9300 and 3800—were obtained in the animals in whom cross circulation was continued for twenty-one and sixteen hours However, the total count for the two animals subjected to cross circulation for three hours was only 8650

TABLE 3

Values for various types of white blood cells at different periods of the experiment (leukopenic and normal cats)

CAT NUMBER	STABS AND SEGMENTED NEUTROPHILES PER C.MM.					LYMPHOCYTES PER C.MM.					EOSINOPHILES PER C.MM.				
	Prior to *X	Start of X	End of X	First after X	End of expt.	Prior to X	Start of X	End of X	First after X	End of expt.	Prior to X	Start of X	End of X	First after X	End of expt.
7	45	7189	5585	3686	175	225	1638	598	76	75	30	273	0	0	0
16	9765	6532	2464	4004	5760	4650	2300	159	396	180	620	276	0	0	0
1	50	11856	14229	10974	60	150	2808	918	472	40	50	936	153	236	0
18	16800	2368	11070	18190	43989	4800	448	861	1926	3311	1680	320	246	428	0
19	0	192	4136	5568	20	100	1608	470	58	20	0	600	94	0	10
20	6110	110	3792	7812	22090	5590	385	118	930	1175	1040	55	39	93	0
23	360	1702	5733	3928	640	216	1443	504	1215	128	12	518	0	0	0
24	8680	162	4895	6375	12831	3220	522	495	825	846	1680	216	0	0	0
25	180	6035	8554	9500	60	70	1445	658	500	40	0	850	0	0	0
26	13650	768	8645	13392	17622	5070	360	475	864	1386	780	72	285	0	0
28	25	2448	7332	5632	77	25	3944	468	768	473	0	272	0	0	0
27	16335	69	6474	15147	7215	11880	2070	1248	2992	3441	1188	161	0	0	0
29	87	2397	1824	759	60	52	1880	528	330	190	210	376	24	0	0
30	8832	945	728	1876	18512	4554	945	616	868	1872	414	189	0	0	0
17	435	4860	5254	7056	448	2088	3420	1775	2450	1088	0	360	0	98	32
8	9750	1085	7110	7425	10285	7995	1736	1620	2376	1573	975	186	90	0	0
Total	91104	48718	97825	121324	139844—	50685	26952	11511	17046	15838	8679	5660	931	855	42
Av. no. per team (2 cats)	11388	6089	12228	15167	17480	6335	3369	1438	2130	1979	1084	707	116	106	5
Av. % of change		*−46.5*	*+7.3*				*−46.8*	*−77.3*				*−34.8*	*−89.3*		
Total of 8 normals				74221	138304				11177	13784				521	0
Av. % of change (normal cats). .					*+86.3*					*+23.3*					*−100*

* Cross circulation.

per cu. mm. Table 3 shows the changes that occurred in the stabs and neutrophiles, the lymphocytes and the eosinophiles. It will be seen that the stabs and neutrophiles showed a percentage drop of 46.5 at the beginning and a rise of 7.3 at the end of cross circulation. Corresponding figures for the lymphocytes were minus 46.8 and minus 77.3, whereas those for the eosinophiles were minus 34.8 and minus 89.3.

After the anastomoses were discontinued and the animals had their own circulations restored, the normal animals showed a prompt increase in the number of white blood cells in the peripheral blood. These normal animals increased the average number of cells per cu. mm. from 11050 immediately after restoration of their circulation to 19337 at the end of the period of observation. In all instances, the observations were continued until the maximum increase had occurred. The percentage increase in the stabs and neutrophiles in the period after cross circulation was 86.3. Corresponding figures for the lymphocytes and eosinophiles were plus 23.3 and minus 100.0.

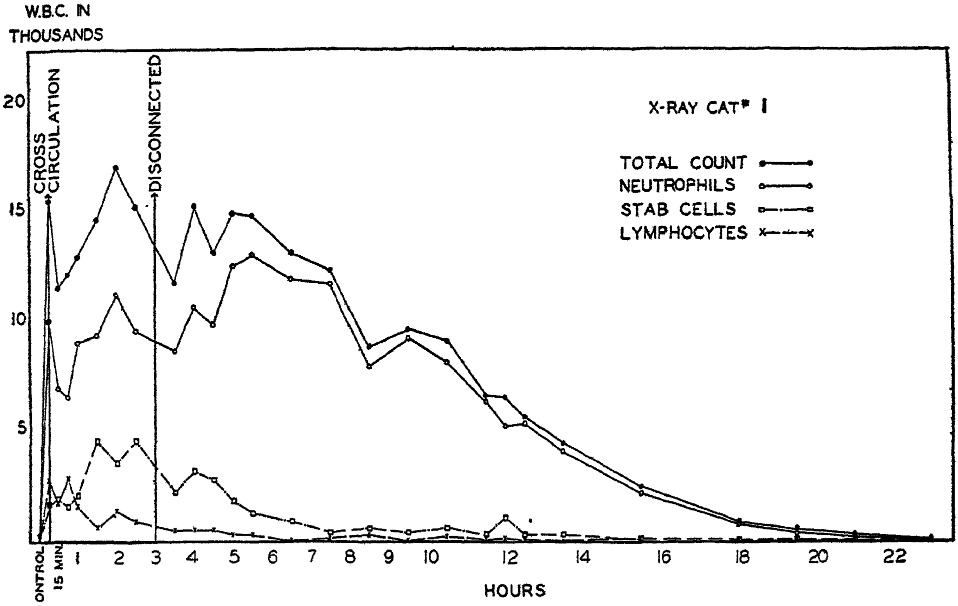

Chart 1. This chart shows the changes in the white blood cell picture of a leukopenic irradiated cat during and following a period of cross circulation with a normal cat.

The time elapsing between the end of the cross circulation and the taking of blood for the first white blood cell studies varied from seventeen to fifty minutes and accounts for the difference in values obtained in the animals in the first determinations made after the cross circulation was stopped, since the white blood cell value dropped in the leukopenic animals and tended to rise in the normal animals when the animals were separated. However, the important figure for our purpose was the height of the white blood cell count at the time when observations were begun.

Reference to table 2 shows that the white blood cells rapidly disappeared in the leukopenic cats after cross circulation was stopped, the original values being reached in a short while. The rate of disappearance of these cells varied from 378 to 1475 white blood cells per cu. mm. per hour. Charts 1 and 2 show the findings in typical experiments.

In one experiment, three normal animals were used for cross circulation with one leukopenic cat. At the end of four hours of cross circulation with the first normal cat, the total number of white blood cells in this normal cat was 5600 per cu. mm. After eight hours of cross circulation with another normal cat, the total white blood cell count of this cat was 14,500 and following ten hours of cross circulation with a third normal animal, the latter had a total white blood cell value of 23,800 per cu. mm. The control figures for all three of these normal cats were between 14,000 per cu. mm. and 15,600 per cu.mm.

Discussion. One of the principal arguments against previous experiments dealing with the length of time the white blood cells remain in the general circulation is that there has been no way of being sure that manufacture of the cells has

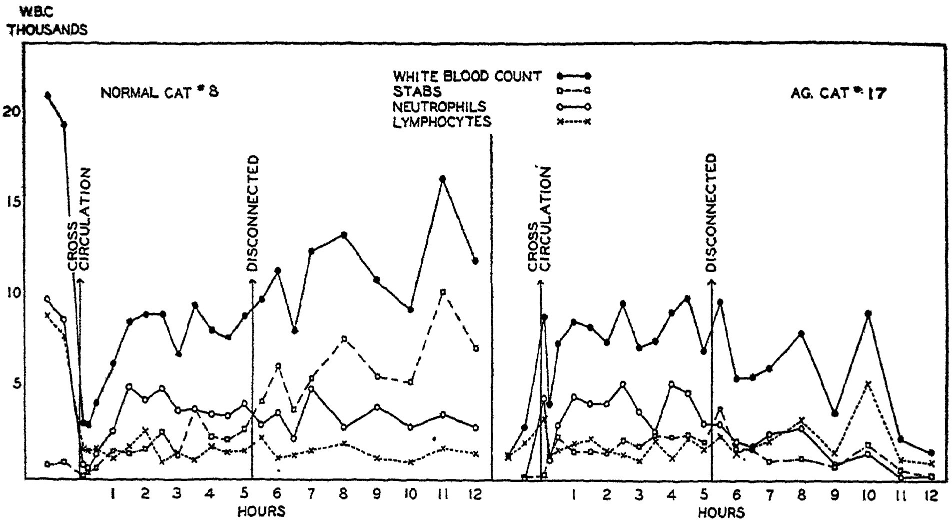

Chart 2. This chart shows the changes in the white blood cell picture of a leukopenic cat and a normal cat during and after cross circulation. The leukopenic cat had infectious feline agranulocytosis.

been stopped or reduced to such a low point that production of cells in these animals could be said not to influence the results. In these experiments we feel that the evidence is in favor of almost total cessation of the manufacture of white blood cells since the white blood cell counts were reduced to very low levels—the average for the nine leukopenic animals being 739 per cu. mm. In only two of the leukopenic animals was the total number of white blood cells above 600 per cu. mm. Hence, it can be assumed that the cells, which were added above the established level of the animals, were not appreciably increased by any production of these cells by the experimental animals. In other words, we can say definitely when the cells were added and when their addition ceased, since no further cells were added after cross circulation was discontinued.

Our results show that the leukopenic cats lost white blood cells, which were given them by cross circulation, at the average rate of 881 cells per cu. mm.

per hour. If a normal cat should use its white blood cells at this rate it would be forced to have them replaced approximately one and one-half times in twenty-four hours, since it has been shown (1) that the average number of white blood cells for the adult cat is 15,000 per cu. mm. However, the assumption that leukopenic animals handle white blood cells as regards their length of life in a normal fashion cannot be made. Indeed, some of our results argue very strongly against such an assumption.

In this connection we may mention the fact that there is a marked leukopenia associated with neutropenia, lymphopenia and eosinopenia at the beginning of cross circulation between a leukopenic and a normal cat. This is in strong contrast to the neutrophilic leukocytosis which was found to occur in the experiments in which a normal cat was connected with another normal cat. The blood changes found at the beginning of cross circulation between a leukopenic and a normal cat are identical with those which we have found (12) are produced by the intravenous injection into normal cats of serum of cats ill with infectious feline agranulocytosis. In this previous work, it was found that the characteristic changes in the white blood cell picture were produced by the intravenous injection of 5 ml. of serum from a sick cat. An attempt was made to discover an explanation for this response of the blood but a satisfactory answer was not obtained. The peripheral blood changes were similar to those seen following foreign protein injections but the omental circulation was not entirely typical of this. However, the rapidity of the reaction was suggestive of some redistribution effect, although it was not pathognomonic of it since such rapid changes have been shown (4) to have been caused by actual destruction of the white blood cells. Whatever the answer may be, we believe the blood changes at the beginning of cross circulation were of the same nature as those obtained with "agranulocytic" serum. It is true that most of the leukopenic animals used in experiments being reported in this paper were irradiated animals whereas those used in the work just referred to were animals with leukopenia due to infectious feline agranulocytosis. However, we suspect that both types of animals react in the same way in this regard, as the one "agranulocytic" animal used in cross circulation showed the same white blood cell changes as the irradiated animals. Whether such a response is common to all types of leukopenia, we cannot say. This effect tended to become less as time went on and, at the end of the period of cross circulation, there was a diminution of the total white blood cell level of 27.5 per cent as compared with 46.5 per cent at the beginning. This increase in the total number of cells was probably due, in a large measure, to increased output of neutrophilic cells by the bone marrow of the normal animal. One indication of this is the fact that the stabs and segmented neutrophiles showed at this time an increase of 7.3 per cent as compared with their number prior to cross circulation.

The diminution of 27.5 per cent in the total number of white blood cells at the end of cross circulation is very difficult to explain since there was an increase of 72.7 per cent when normal animals were anastomosed. It is of some interest to attempt to analyze this discrepancy. For purposes of discussion we shall

assume first that the individual animal consumes white blood cells at a given rate regardless of their age. We shall make the further assumption that this rate is 881 cells per cu. mm. per hour and that it holds for normal as well as leukopenic animals. Then, if we consider that the bone marrow of only one animal is functioning in the experiments where a leukopenic animal is anastomosed with a normal animal, we must arrive at the conclusion that the rate of use of white blood cells under these conditions will be 1762 (2 × 881) per cu. mm. per hour. In other words, the loss of white blood cells per unit of bone marrow is twice the normal. Thus, 881 cells per cu. mm. would be lost in excess of normal each hour. Hence if the normal animal is able to increase its white blood cell count 72.7 per cent, the sum total of leukocytes in the combined peripheral blood of the two animals should, at the end of cross circulation, be equal to the combined total white blood cell count prior to cross circulation plus 72.7 per cent of the number of white blood cells present in the normal cat prior to cross circulation and minus 881 white blood cells per cu. mm. per hour of cross circulation destroyed by the leukopenic animal. The average length of cross circulation in this group of experiments was 7.4 hours. Applying this reasoning to combined figures of the eight separate cross circulation experiments carried out the following data are pertinent:

(1) Combined total white blood cell counts (8 normal plus 8 leukopenic cats) prior to cross circulation	154050
(2) Combined total white blood cell counts of 8 normal cats prior to cross circulation	149000
(3) White blood cells destroyed per leukopenic animal per cu. mm. per 7.4 hours (881 × 7.4)	6520
(4) Total cells destroyed by 8 leukopenic cats	52160

Then at the end of cross circulation the expected total number of white blood cells for the entire group of eight normal and eight leukopenic cats would be 154050 plus 108423 (149000 × 0.727) minus 52160 or 210323 white blood cells. However, at the end of the cross circulation the combined total white blood cell count was only 111750, leaving a deficit of 98573 cells which is unexplained. This deficit becomes even greater if we assume that white blood cells live a certain period of time and do not die until that age is reached, for, under these conditions, the rate of loss in cross circulation between a leukopenic and a normal animal would be no greater than in the case of a normal animal alone. We doubt this being the case but have no proof that it is not true. Further, we have no proof that the normal animal uses white blood cells as rapidly as the leukopenic animal. If this is not true, the discrepancy would become even greater.

This marked difference between the number of cells actually in the peripheral blood and the number that theoretically should be present is capable of several explanations. One of the first possibilities that arises is with reference to the presence of some leukotoxic substance or substances in the blood of the leukopenic animals. If such were the case, the same or a similar substance must have been present in the "agranulocytic" animal. The fact that manufacture of cells proceeded in a normal fashion after cross circulation was stopped argues against

but does not refute the possibility that small amounts of some leukotoxic substance or substances were present in the circulating blood of leukopenic animals. On clinical grounds, it would seem logical to assume that leukopenia in general was associated with such a substance since it is known that the white blood cells of patients with leukopenia cannot be elevated for any period of time by transfusions with blood containing normal numbers of cells. If this is true, one could explain it by considering that normal animals produced enough of such a substance to destroy white blood cells at a normal rate and that this material was neutralized as it reacted with white blood cells. If this were the case, leukopenic animals would have an excess of leukotoxic material. This would readily explain a more rapid disappearance of white blood cells in leukopenic than in normal animals. However, we have no proof that this situation exists. Of course, it is entirely possible that irradiation results in the production of some leukotoxic substance and that this is different from any leukotoxic substance that may be associated with leukopenia due to other mechanisms.

Another theoretical explanation is that the leukopenic animals had consumed the white blood cells that were normally in the tissues and, therefore, removed those given them from the blood more rapidly than normal. If this had been true, it should have been possible to have noted a dimunition in the rate of disappearance from the peripheral blood of the cells in those animals in whom cross circulation was maintained for long periods of time as compared with those in whom cross circulation was continued for short periods of time. Reference to table 2 shows that this was not the case. Actually the lowest rate for disappearance of the white blood cells—378 per cu. mm. per hour—occurred in an animal that was subjected to cross circulation for only two and one-half hours. The most rapid rate of disappearance was found in cat 31. In this animal cross circulation was maintained for twenty-two hours and, in addition to this, three normal cats were used in cross circulation so as to avoid the leukopenia which seemed to be the result of a greater demand for cells than the bone marrow of our normal animals was capable of supplying.

Still another explanation for the discrepancy between the white blood cells present and those that theoretically should be present is temporary bone marrow exhaustion of the normal animal connected to the leukopenic animal. In other words, the demands for white blood cells under these conditions *may* have been greater than the bone marrow of the normal animal could supply. That bone marrow exhaustion may occur is suggested by the fact that leukopenia may develop in some fulminating infections. That temporary bone marrow exhaustion may have developed in our experiments is indicated by the results obtained when three normal cats, rather than one, were used for cross circulation. In this experiment the level of the white blood cells was raised each time a new animal was used, the level at the end of use of the first normal being 5600 whereas the level following the use of the second animal was 14,500 and that following use of the third cat was 23,800 per cu. mm. Only one experiment of this sort was done and, of course, no conclusions can be drawn from it. Much work would be required to answer the question as to the possibility of bone marrow exhaus-

tion. However, we can state that, if such exhaustion did exist, it was not permanent since our normal animals were able to show an increase of 75.0 per cent in the total number of white blood cells when cross circulation was discontinued. Other possible explanations exist but we shall not discuss them. We regret that we are unable to indicate the correct explanation at this time.

The question naturally arises as to whether the reported rate of disappearance of the white bood cells represents the rate at which they are destroyed. We are inclined to feel that such is the case for, if it were not, then cells would accumulate in large numbers in certain areas. This would result in constantly increased numbers of cells in the tissues with increased age. Such is known not to occur. Further, actual study of sections from tissues of various organs of the body has failed to reveal any excessive accumulation of these cells. (14) Also, if the cells were leaving the circulation only to return and be used again, one would have a right to expect that they should have returned to the peripheral circulation of our animals.

Additional evidence of the same nature was obtained by observing the omental circulation of cat number 25 under the microscope during an entire experiment. Prior to cross circulation the white blood cell count of this animal was 250 per cu.mm. At this time, no white blood cells were visible in the vessels of the omentum. Many white blood cells appeared in these vessels soon after cross circulation was begun. They continued to be visible in these vessels throughout the period of cross circulation. Following discontinuation of the anastomosis, there was a gradual disappearance of the white blood cells in the omental vessels associated with the steady diminution of these cells in the peripheral blood. In other words, the number of cells in the para-capillaries of the omentum showed excellent correlation with the number in the peripheral blood.

The depreciation in the lymphocyte level at the end of cross circulation was probably due to the relative increase in the stabs and neutrophiles since this is a well recognized reaction. Even though the total white blood cell count was diminished 27.5 per cent at the end of cross circulation there was an average percentage increase of 7.3 in the stabs and neutrophiles at this time. It should be noted that there was a corresponding increase of 132.3 per cent in the stabs and neutrophiles and a decrease of 50.4 per cent in the lymphocytes at this time in the experiments where two normals were used.

The marked diminution in the eosinophiles during cross circulation can be explained as the result of the operative procedures. This is a well recognized response and has been commented upon previously (9).

Attention should be called to the fact that basophiles and monocytes are present in such small numbers in the blood of the cat (1) that our observations cannot cover them. Further, the eosinophile almost completely disappeared during cross circulation (presumptively as the result of the operative procedure) and hence was not involved in the cells that disappeared after cross circulation was discontinued. Actually, the neutrophilic segmented and stab cells were the ones that were mainly concerned. The observations reported in a separate communication by Adams, Saunders and Lawrence (2) are more pertinent as regards

the rate of disappearance of the lymphocyte although this cell was present in sufficient numbers in some of the experiments to indicate a trend in the same direction as that of the neutrophile.

The great discrepancy between our findings as regards the life of the white blood cells in leukopenic animals and those of investigators using tissue culture is easy to understand. In tissue culture, the cells are not called upon to perform any duties and are, to all intents and purposes, in storage. Hence the wear and tear on them is minimal. The situation is comparable to the difference between the life of a motor kept in storage and that of one kept in active use.

Our results (2) in studies of the lymphocyte indicate a more rapid replacement for these cells than has been found in the present work, which deals largely with neutrophilic cells so that the total turnover is probably greater than one and one-half each day. We are not sure, as indicated above, that the rate of disappearance of cells in the leukopenic animal is the same as in the normal animal. If this should be the case, it would indicate a rapid turn-over of white blood cells—that is, approximately twice in 24 hours. We are inclined to feel that the turn-over is a fairly rapid one even in normal animals and, therefore, suggest that the white blood cells are in the blood largely as a means of transportation and that they rapidly pass from it to the various body tissues where they are soon destroyed. What their functions are in these tissues cannot be stated in full. However, their rapid use indicates that they may be much more important in body metabolism than is generally assumed. While they may carry out important functions in the blood, they probably are functionally much more important in the various tissues of the body.

If this hypothesis is correct it indicates a wide difference between the red blood cell and the white blood cell since the former cell remains in the peripheral blood throughout most of its life of approximately one hundred and twenty days. This hypothesis makes it necessary, also, to assume a very much greater production of white blood cells than would be assumed on the basis of their numerical values in the blood. This is in agreement with the known fact that there are large amounts of myeloid tissue and lymphoid tissue in the body. Actually the amount of myeloid tissue is greater than that of erythroid tissue, the average ratio in the human being 3.6:1 according to Osgood (17). This ratio takes no account of the lymphoid tissue and the connective tissue that forms monocytes and, accordingly, must be too low if we consider all of the white blood cell forming tissues. If we assume a leukopoietic erythroid ratio of 4:1, this certainly will be low rather than high. If we make the additional general assumptions: 1, that the red blood cells of the cat live on the average 100 days; and 2, that the ratio of volume of white blood cells to red blood cells in the circulating blood is 1:80, we can arrive at some conclusion as to the expected life of the white blood cell in the cat. Thus, if the leukopoietic tissue and the erythroid tissue have an equal production rate, there should be four times as many white blood cells as red blood cells provided both types of cells have the same length of life. Actually, the volume of the white blood cells is only approximately one-eightieth that of the red blood cells. Now, if the production rate is held to be equal for both types

of tissue, the only way this difference can be accounted for is by a difference in the life span of the two types of cells. Thus, the expected life of a white blood cell on this basis would be 0.3 of a day (100 ÷ 320) or 7.2 hours. This is admittedly a very rough approximation but it is perhaps significant that it does result in a figure which is not far removed from the one we have found—namely, sixteen hours. In other words, both the expected life of a white blood cell and that actually found in our experiments are a matter of hours rather than days.

CONCLUSIONS

The average rate of disappearance of white blood cells in the cat *under* the *conditions* of these experiments is 881 cells per cu. mm. per hour.

The hypothesis is advanced that the white blood cells of the cat may be replaced about one and one-half times in twenty-four hours. It is assumed that the white blood cells use the general circulation as a means of transportation. This does not mean that they are functionally inactive while in transit but suggests that their more important functions are performed in the body tissues other than the blood.

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OUTPUT OF LYMPHOCYTES IN CATS INCLUDING STUDIES ON THORACIC DUCT LYMPH AND PERIPHERAL BLOOD[1]

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The scientific literature of the past seventy years contains many qualitative and quantitative determinations of the cellular content of thoracic duct lymph. Since the time such investigators as Lesser (15), Colin (6), Zawilski (25), Merunowicz (16), Heidenhain (10) and Winternitz (23) first recorded the rate of flow of lymph from the thoracic duct of various animals, interest has been focused on its contents.

The work of later investigators has shown a diminution in the number of lymphocytes in the blood following exclusion of the thoracic duct lymph from the circulation—Biedl and von Decastello (2), Selinoff (21), Crescenzi (7), Rous (18, 19), Bunting and Huston (5), Lee (14), and Blalock et al. (3).

However, Bloom (4) stated that the thoracic duct was not the main avenue for the entrance of lymphocytes into the general circulation. Sanders, Florey, and Barnes (20), working on cats and rabbits, held that "the fall of lymphocytes in the blood bears no relation to the number lost in the escaping thoracic lymph". They observed a fall from the normal of 5,000 lymphocytes per cmm. to 2,000 per cmm. in peripheral blood soon after a thoracic duct cannula was tied in place, and stated that correlation between the number of lymphocytes in the blood and the number in the thoracic duct was impossible because of the unstable blood picture caused by operation.

We became interested in this problem because of studies which we were making with reference to the life cycle of the white blood cells. Since there was some conflict in the results obtained by the investigators in this field, we felt that we should make our own observations. Accordingly, a series of experiments dealing with thoracic duct lymph of the cat has been performed. This report deals with our findings and, as such, gives our results with reference to: 1, the rate of lymph flow in cats; 2, the average hourly output of lymphocytes per kilogram of body weight in the thoracic duct of normal cats; 3, the effect on the peripheral blood caused by removal of thoracic duct lymph; 4, the effect of irradiation on lymphocyte production in the lymph of cats; 5, the effect of the virus disease, infectious feline agranulocytosis on lymphocyte production in the lymph of cats; and 6, the identity of cells as found in normal thoracic duct lymph.

METHODS. The data which have been used in this paper have been collected from observations made on forty cats: twenty-one normal animals with thoracic

[1] The expenses for this work were defrayed in part by a grant from the John and Mary R. Markle Foundation.

[2] Serving in the armed forces.

duct cannulation, ten normal animals operated upon but not cannulated (controls), five irradiated animals and four agranulocytic animals. (Cats referred to hereafter as agranulocytic are cats sick with infectious feline agranulocytosis—Lawrence and Syverton (12).) The cats which were selected were young and weighed 1.93 kilograms on the average.

One group of twenty-one normal animals was treated as follows: Four hours after the cats were fed 40 cc. of light cream, they were anesthetized by an intraperitoneal injection of dial and urethane. Thereupon the thoracic duct was exposed in the following manner: an area over the upper chest and thoracic inlet was shaved and an incision 2 to 3 cm. long was made slightly to the left of the midline. The pectoral and clavotrapezius muscles were dissected free down to the junction of the jugular and subclavian veins. The thoracic duct was exposed and ligated next to its venous junction or junctions. The duct was nicked with a pair of fine scissors, and a glass cannula was inserted and tied in place. The cat was then rotated to a prone position with the head and forelegs supported. The lymph was allowed to drip by gravity into a collecting burette graduated in tenths of a cubic centimeter. At intervals of fifteen minutes white cell counts were done in duplicate on lymph pipetted directly from the cannula. The amount of lymph collected during each fifteen minute period was recorded. Every one-half hour a white cell count and a smear were made on the peripheral blood obtained from the marginal ear vein. The cat was kept alive as long as the flow of lymph remained sufficiently great to permit accurate measurement of flow. Water balance was maintained by giving hypodermic saline injections, the total amount given being equal to the amount of lymph withdrawn. Normal body temperature was sustained by the use of an electric lamp. Usually after six or seven hours the experiment was terminated and the animal sacrificed. Thirteen of these animals had only one pre-operative white blood cell count. It was realized that it was not statistically accurate to compare this single value with multiple post-operative counts. Accordingly, the remaining eight animals of this group had a three hour pre-operative control period during which time six white blood cell counts and six differential counts were made on the peripheral blood.

A second group of ten normal cats had the same procedures carried out as in the first group of twenty-one normal cats except for actual cannulation of the thoracic duct. In other words, the thoracic duct was exposed, dissected free and then packed with moist sponges. The trauma inflicted was equivalent to that inflicted on the animals whose thoracic ducts were cannulated. Since no lymph was removed from these animals, no saline injections were given.

A third group of five normal cats was irradiated. Each cat was given 1,600 roentgen units (with the viscera protected by lead shields) to each of the following regions: 1, to head, forelegs and cervical vertebrae; 2, to tail, hindlegs and pelvic girdle; 3, to upper sacral vertebrae and lumbar vertebrae. Four to five days following the above exposure, the cats were used in thoracic duct cannulation experiments as described for the first group of twenty-one cats.

A fourth group of four cats was infected with the virus of infectious feline

agranulocytosis, according to the methods of Lawrence and Syverton (12), and at the height of this disease were used for cannulation experiments, as described for animals of the first group.

TABLE 1

Thoracic duct lymph data

TYPE OF ANIMAL	TOTAL OUTPUT OF LYMPHOCYTES IN MILLIONS PER KILOGRAM PER HOUR			AVERAGE NUMBER OF LYMPHOCYTES PER CU. MM. OF THORACIC DUCT LYMPH	NUMBER OF CC OF LYMPH PER HOUR
	Minimum	Maximum	Average		
Normal	9.6	92.6	35.5	14,156	5.02
Irradiated	3.1	10.4	5.6	2,011	4.75
Agranulocytic	0.5	39.7	15.6	3,932	4.11

TABLE 2

Results of thoracic duct cannulation of twenty-one normal cats

EXPERIMENT NUMBER	DURATION IN HOURS	LYMPH FLOW NUMBER OF CC. PER HOUR	AVERAGE LYMPHOCYTE COUNT OF THORACIC DUCT LYMPH PER CU. MM.	AVERAGE WHITE BLOOD CELL COUNTS PER CU. MM.	AVERAGE NUMBER OF LYMPHOCYTES IN BLOOD PER CU. MM.	THORACIC DUCT LYMPHOCYTES IN MILLIONS PER KILOGRAM PER HOUR
1	3.0	1.80	24,625			24.5
2	4.0	7.12	9,471			35.6
3	2.5	2.84	15,842			31.4
4	2.0	5.75	7,219	22,650	2,491	29.8
5	7.0	3.14	15,933	15,480	1,548	33.6
6	6.0	3.02	4,288	23,223	3,065	9.6
7	8.0	6.05	28,779	22,182	2,285	92.6
8	6.0	4.90	11,690	26,584	2,525	27.9
9	6.0	3.72	11,748	45,207	2,622	29.6
10	6.5	4.08	12,321	18,635	3,727	24.2
11	6.0	6.23	14,850	16,592	1,377	40.3
12	7.0	6.77	29,587	39,893	4,787	86.5
13	6.0	5.22	21,800	31,960	2,205	44.4
14	6.0	2.83	12,655	42,880	4,803	19.0
15	6.0	6.44	11,183	19,775	1,700	37.6
16	6.0	4.16	20,602	17,350	3,643	50.3
17	6.0	6.88	7,900	28,166	2,253	19.2
18	6.0	4.94	7,805	38,066	2,474	21.7
19	6.0	6.93	5,208	42,500	2,762	13.6
20	6.0	7.28	7,600	37,916	3,526	35.3
21	6.0	5.36	16,166	17,833	1,908	39,1
Average		5.02	14,156	26,160	2,761	35.5

RESULTS. Most of the results are shown in the tables and charts. It will be seen that the lymphocyte output of the thoracic duct of the normal cat is a very variable factor. The range is from 9.6 to 92.6 $\times$ 10^6 per kilogram per hour. Study of table 2 shows that there is no close correlation between the output of

lymphocytes per hour in the lymph and either the total white blood cell count or the number of lymphocytes in the blood during the period of cannulation.

The amount of lymph flow per hour is subject to wide variations also. The range in twenty-one normal cats was 1.80 cc. to 7.28 cc. per hour. Variations of approximately the same magnitude were found in the irradiated and the agranulocytic cats as seen in tables 3 and 4.

Cannulation of the thoracic duct was followed by an appreciable diminution in the number of the lymphocytes in the peripheral blood. However, practically

TABLE 3

Results of thoracic duct cannulation of cats made leucopenic by irradiation

EXPERIMENT NUMBER	DURATION IN HOURS	LYMPH FLOW NUMBER OF CC. PER HOUR	AVERAGE LYMPHOCYTE COUNT OF THORACIC DUCT LYMPH PER CU. MM.	AVERAGE WHITE BLOOD CELL COUNTS PER CU. MM.	AVERAGE NUMBER OF LYMPHOCYTES IN BLOOD PER CU. MM.	THORACIC DUCT LYMPHOCYTES IN MILLIONS PER KILOGRAM PER HOUR
32	3.0	3.80	1,683	1,542	231	4.0
33	4.5	3.00	1,600	1,066	607	3 2
34	6.0	4.76	1,122	700	262	3.5
35	6.0	4.93	3,300	2,212	619	6.9
36	6.0	7.23	2,352	3,279	823	10.4
Average		4.75	2,011	1,760	508	5.6

TABLE 4

Results of thoracic duct cannulation of cats during leucopenic stage of infectious feline agranulocytosis

EXPERIMENT NUMBER	DURATION IN HOURS	LYMPH FLOW NUMBER OF CC. PER HOUR	AVERAGE LYMPHOCYTE COUNT OF THORACIC DUCT LYMPH PER CU. MM.	AVERAGE WHITE BLOOD CELL COUNTS PER CU. MM.	AVERAGE NUMBER OF LYMPHOCYTES IN BLOOD PER CU. MM.	THORACIC DUCT LYMPHOCYTES IN MILLIONS PER KILOGRAM PER HOUR
37	6.0	6.13	5,512	511	467	16.5
38	5.0	2.82	1,700	225	195	5.5
39	5.3	5.02	8,801	1,395	1,122	39.7
40	4.0	2.47	435	200	120	0.5
Average		4.11	3,932	583	476	15.6

the same effect on the peripheral blood was produced by carrying out all of the procedures incident to cannulation but with omission of cannulation. Charts 1 and 2 show this clearly.

The cats that were leukopenic as a result of irradiation had very small numbers of lymphocytes in the thoracic duct lymph and a low output of lymphocytes per kilogram per hour, the average being 5.6×10^6 per kilogram per hour.

The agranulocytic cats had low values for lymphocytes in the thoracic duct lymph but they were not so low as in the irradiated animals. The average figure for lymphocytes in thoracic duct lymph of the agranulocytic cats was 3,932 cells

per cmm. as compared with 2,011 cells per cmm. for the irradiated cats. The output of lymphocytes per kilogram per hour was distinctly greater in the agranulocytic cats than in the irradiated cats, the average being 15.6 $\times$ 10^6 per kilogram per hour. In this connection, it should be noted that the leukopenia was more profound in the agranulocytic cats and the average total white blood cell value in this group was 583 as compared with 1,760 per cu.mm. for the irradiated cats.

In normal cats the average hourly output of thoracic duct lymph was 5.02 cc. This figure is based on twenty-one normal experiments with cats fed 40 cc. of light cream During the experiments it was noted that the rate of lymph flow varied widely as had been found by numerous previous investigators in this

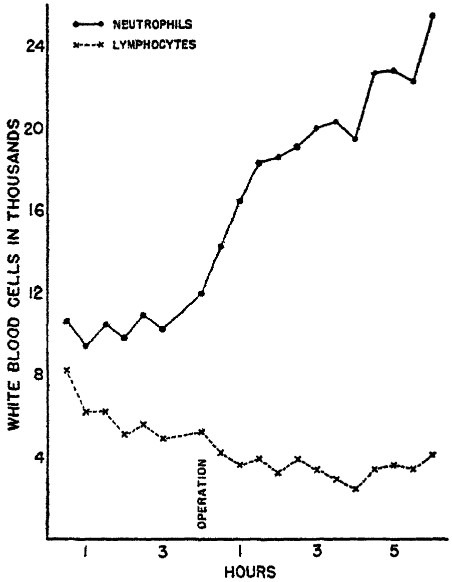

Chart 1

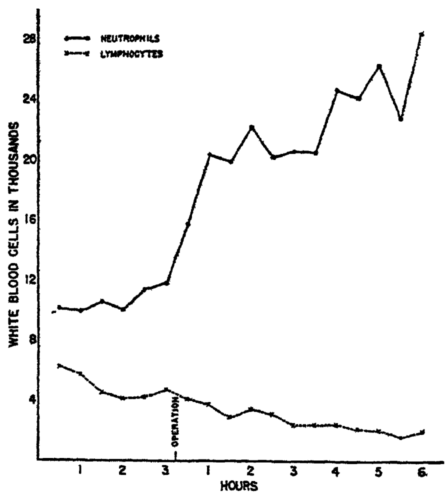

Chart 2

Chart 1. Control experiments 10 normal animals
Chart 2. Thoracic duct cannulations 8 normal animals

field, among others, Drinker and Yoffey (9). Twenty-five smears of thoracic duct lymph from the normal cat were examined. The averages of these differential counts was: eosinophiles 0.24 per cent, neutrophiles 1.12 per cent, small lymphocytes 92.80 per cent, intermediate lymphocytes 5.12 per cent, large lymphocytes 0.64 per cent, and unclassified 0.12 per cent. A few red blood cells were constantly found in the thoracic duct lymph.

Discussion. The wide variations which we found in the output of lymphocytes in the thoracic duct differ from the findings of Rous (18, 19) but are in agreement with the findings of Drinker and Yoffey (9). It should be pointed out that there was not only a wide variation from cat to cat but, also, marked variations in the same animal from hour to hour.

If we take the average figure of 35.5 $\times$ 10^6 lymphocytes per kilogram per

hour as the average output of lymphocytes by way of the thoracic duct we can estimate the daily replacement of these cells by way of thoracic duct lymph. The normal cat probably has a blood volume representing 6.5 per cent to 8.0 per cent of its body weight (Welcker (22) and Robscheit-Robbins (17)). Thus, the average number of cubic millimeters of blood per kilogram of cat is 72,500. Ackart, Shaw and Lawrence (1) found that the average adult normal cat had 4,800 lymphocytes per cu. mm. of blood. Accordingly, a normal cat can be considered as having 348×10^6 lymphocytes per kilogram of body weight at any one time. Eight hundred and fifty-two $\times 10^6$ ($= 35.5 \times 10^6 \times 24$) lymphocytes per kilogram will be delivered to the blood in twenty-four hours by way of the thoracic duct lymph. In other words, enough lymphocytes enter the blood by way of the thoracic duct lymph to replace those present in the blood 2.4 times in twenty-four hours. This figure agrees closely with that of Yoffey (24), who obtained for the dog a corresponding figure of 2.06. It is also in close agreement with the figures for cats of 0.5 to 3 obtained by Sanders, Florey and Barnes (20). Inasmuch as thoracic duct lymph represents only a part of the lymph that gets into the blood, these figures are probably too low if used to represent the actual daily replacement of lymphocytes in the blood

It is interesting to speculate whether these figures represent replacement of new lymphocytes via the thoracic duct or whether an acutal circulation of lymphocytes occurs between blood and lymph. Drinker and Yoffey (9) calculated that, "of the lymphocytes entering the blood by way of the lymph, one in thirty-two was returning to the blood after having entered the lymph, while the other thirty-one were newly formed cells." There is good support for the circulation of lymphocytes which, although demonstrable, is not of great significance when one considers the total lymphocyte replacement occurring via the thoracic duct. We must, therefore, come to the conclusion that the life span of the normal lymphocyte of the cat is not more than ten to twelve hours.

Our results do not give support to those who hold that the diminution in the number of lymphocytes in the blood, which follows ligation or cannulation of the thoracic duct, is due wholly to failure of these lymphocytes to enter the systemic circulation. Since Biedl and von Decastello (2) first ligated the right lymphatic duct and thoracic duct in dogs and noted a drop in blood lymphocytes, interest has been focused on the effect of exclusion of lymph from entering the systemic circulation. The observation that an absolute lymphopenia occurs has been substantiated by: Davis and Carlson (8), Bunting and Huston (5), Lee (14), Kindwall (11), Drinker and Yoffey (9) and Blalock (3). By comparison of charts 1 and 2 it can be seen there is only a slight difference between the normal fistula cats and the control animals. (This difference is actually only 5 per cent.)[3] It should be pointed out that the leukocytosis in the two groups compares very closely (51.8 and 46.0 per cent). From these data it would seem that the drop

[3] A statistical analysis of our figures has been made by J. A. Rafferty, who by the application of an Analysis of Variance, found that the control period precedent was followed within the ten per cent probability for all points except the last. In other words, his conclusions are in conformity with ours.

in blood lymphocytes following cannulation of the thoracic duct was not solely due to the formation of a thoracic duct fistula but possibly due to a neutrophilic leukocytosis with a concomitant lymphopenia. The majority of investigators in this field have obtained a neutrophilic leukocytosis following thoracic duct cannulation or ligation. One exception is found in the report of Bunting and Huston (5) who cite two experiments of the ligation of the right lymphatic and thoracic ducts of rabbits in which they obtained no rise in the total white blood cell count following operation and obtained a drop in blood lymphocytes. It is our opinion that the drop in blood lymphocytes following thoracic duct cannulation is due, only in part, to the formation of a thoracic duct fistula but in part to the depressant action of a neutrophilic leukocytosis on the lymphocytes of the peripheral blood. This depressant action is a well known clinical fact, for commonly a neutrophilic leukocytosis is accompanied by a lymphopenia. How much each of these mechanisms is responsible cannot be stated on the basis of our data. If our calculations are correct one would expect a diminution in the lymphocytes of approximately 50 per cent, if the thoracic duct lymph was the sole source of lymphocytes during a period of six hours of cannulation of the thoracic duct. The real test of the effect of cannulation of the thoracic duct would be to continue the cannulation for much longer periods of time. Such experiments have not been carried out.

The differences in the lymphocyte output in the irradiated and agranulocytic animals are rather striking. While the number of animals used is not great we suspect that these differences are real. It seems to us that the higher figures in agranulocytic animals in the face of distinctly lower total white blood cell counts are significant. We have no entirely satisfactory explanation but would like to raise the question as to whether the answer is not to be found in pathological changes in the lymph nodes of the irradiated animals. The changes in the irradiated animals were, no doubt, more marked than those in the agranulocytic animals. We did not make any detailed observations in this connection but we know from studies of the pathological changes in agranulocytic animals (Lawrence et al., 13) that the changes in the lymph nodes are not so great as in heavily irradiated tissues.

SUMMARY

1. Observations were made on the cell content of thoracic duct lymph of twenty-one normal cats. The average lymphocyte output was found to be 35.5×10^6 lymphocytes per kilogram per hour—a figure which could account for the replacement of blood lymphocytes every ten to twelve hours.

2. It was found that the diminution in the number of lymphocytes in the blood during thoracic duct cannulation for six hours was due, only in part, to loss of lymphocytes in lymph passing out in the fistula.

3. The output of lymphocytes in the thoracic duct lymph was distinctly less in irradiated animals than in agranulocytic animals in spite of the fact that the average total white blood cell count was distinctly less in the agranulocytic cats.

4. The average lymph flow was found to be 5.02 cc. per hour in normal cats.

5. Differential counts of thoracic duct lymph yielded the following results: eosinophiles 0.24 per cent, neutrophiles 1.12 per cent, lymphocytes 98.56 per cent, unclassified cells 0.12 per cent. A few red blood cells were present constantly.

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VASCULAR RESPONSES OF THE NASAL MUCOSA TO THERMAL STIMULI WITH SOME OBSERVATIONS ON SKIN TEMPERATURE [1]

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The present study seeks to extend our knowledge of the vascular responses of the human nasal mucosa to thermal stimulation of the skin. In a classical series of papers published between 1919 and 1921, Mudd, Grant and Goldman (1, 2, 3) believed that their researches dispensed with the notion that chilling of the body surface causes nasal turgescence. We shall show that such nasal turgescence may, and frequently does, occur. We were further interested in following up the work of Spiesman (4) and Spiesman and Arnold (5) who claimed that the nasal responses in common cold resistant subjects were different from those in hypersensitive subjects. Such an observation, if confirmed, clearly points a way to further research into the physiological factors predisposing to the common cold and other respiratory disturbances.

METHODS. 1. Temperature measurements of the skin and of the mucosa of the anterior portion of the inferior turbinate were made with constantan-copper thermocouples which consisted of two strands of no. 26 wire soldered with a small silver bead. The diameter of the bead was about 1 mm., and the remainder of the unit about 2 mm. The thermocouple was held in place on the turbinate by means of a device recommended by Hertzman and Dillon (6); this consisted of a system of rods and universal joints which in turn were attached to a plaster of Paris cast of the cranium. A potentiometric method of measuring the temperature was used and was accurate to about 0.2°C.

2. Measurements of changes in the volume of the nasal space were made with a rubber balloon in the deflated state 4.8 cm. long and 1.8 cm. in maximum diameter. Measurements on sagittal sections of human heads show that the tip of the balloon must have extended to about the level of the posterior half of the middle turbinate. After insertion of the balloon it was inflated and deflated a number of times with an internal pressure of 20 mm. mercury, in order to remove any possible folding-over, and the actual recording of changes in volume made with a very small residual positive pressure in the balloon. The balloon was connected with a standard plethysmograph system, the oscillations of a rubber membrane being optically recorded. The changes in nasal volume mentioned in this paper are given in arbitrary units referred to an initial

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base line. Such a plethysmographic system is very sensitive, showing not only gross changes in volume of the nasal cavity, but also the arterial volume pulse.

3. During the experiments in which nasal temperatures and volume changes were being simultaneously recorded, the subject breathed through the mouth. This was made necessary due to the complete blocking of one nostril by the balloon, and the partial blocking of the other nostril by the thermocouple. This had the advantage, however, of materially reducing respiratory effects, which always must be considered. At this point, we may say that in the course of our experiments we have satisfied ourselves that respiratory changes cannot account for the phenomena to be described.

4. With a view to determining whether or not there is a relation between digital blood flow and physiological changes in the nasal mucosa, a large number of experiments with the standard venous occlusion finger plethysmograph, also optically recorded, was performed. Our efforts in this direction were in the main unsuccessful. We are unimpressed with this apparatus as a really quantitative instrument. It will reveal gross changes in finger blood flow, but in our experience is almost useless for following changes of a more refined sort. Not only do the apparent changes in flow from moment to moment, due to many and frequently obscure causes, complicate the interpretation, but even the correct method of determining the initial slope of the volume-time curve at high rates of flow is open to question. In spite of much pondering over the matter we are unable to find the theoretical justification for Burton's (7) method of determining the slope line at high rates of flow.

5. The experimental studies were made in a room in which the temperature could be controlled over a range of about 20°C (9°–29°C). The relative humidity varied from about 70 per cent at the lower temperatures to about 80 per cent at the higher temperatures.

6. One hundred and forty-five experiments were performed on 37 subjects. About one-third of the subjects were medical students, about one-fourth were clinic patients, and the remainder were staff members of the medical school departments. The subjects were classified as follows: *a*, common cold resistant (averaging less than one cold per year), 2 subjects; *b*, moderately susceptible to colds (averaging 2 to 3 colds per year), 15 subjects; *c*, very susceptible to colds (averaging 4 or more colds per year), 7 subjects; *d*, sufferers from allergic rhinitis, 8 subjects, and *e*, miscellaneous, including one subject with a mild Raynaud syndrome, one with a severe allergic gastroenteritis, two with a physical allergy to cold and one with an atopic dermatitis, 5 subjects.

RESULTS. A. *Effects of local chilling and warming.* Figure 1 shows characteristic effects of hot (45°–50°C.) and cold (13°–15°C.) stimulation of a local area of the body upon the temperatures of the nasal mucosa and finger. The room temperature was 20°C. The noteworthy changes in the nasal temperature were: 1, a pronounced drop upon application of the cold stimulus; 2, incomplete recovery while the stimulus was still being applied; 3, a drop upon removing the cold stimulus; 4, a drop upon application of the hot stimulus; 5, recovery to a

point beyond the original level while the hot stimulus was still being applied; 6, a drop upon removal of the hot stimulus.

It will be seen that there is a fairly close correspondence between the nasal and finger temperatures. The sudden drops occurring with each change of stimulation may be readily explained on the basis of primary vasoconstrictor reflexes. The secondary changes, in the case of the cold stimulus, exemplify adaptation, while in the case of the hot stimulus suggest gradual vasodilatation.

The results described above are typical, but not invariable. Thus, adaptation to the cold stimulus does not always occur, nor is vasodilatation due to the hot

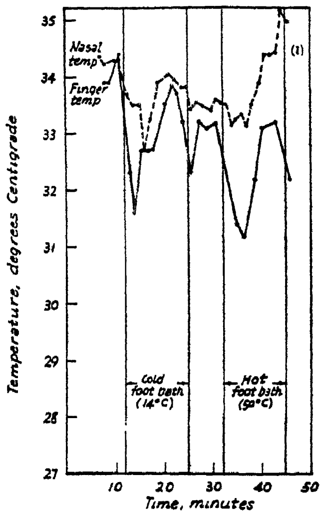

Fig. 1

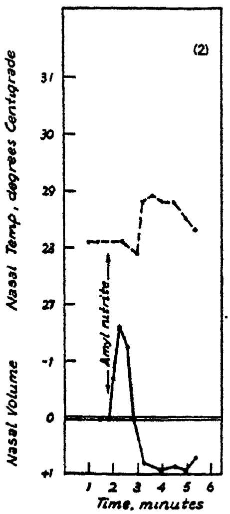

Fig. 2

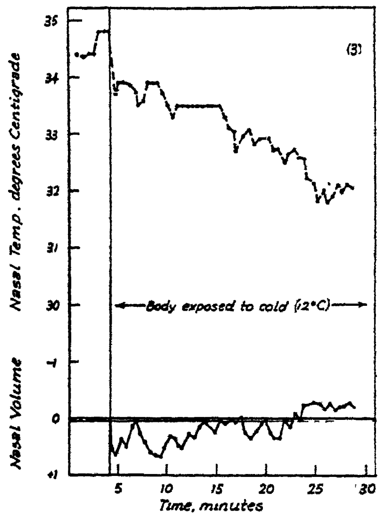

Fig. 3

Fig. 1. Showing the typical effects of local chilling and warming upon the nasal and finger temperatures. See text for further discussion.

Fig. 2. The effect of amyl nitrite, inhaled orally, upon nasal temperature and nasal volume. The subject had been exposed to a cold environment prior to the administration of the drug.

Fig. 3. Showing the gradual decrease in nasal volume (i.e., swelling of the nasal mucosa) during prolonged chilling of the body. See text for further discussion.

stimulus always found. However, in only one subject of our entire series has any important deviation from the general rule been observed. We shall come back to this point later.

B. *The effect of drugs upon the nasal temperature and volume.* Jackson (8), in a beautiful series of experiments on dogs studied the effects of various pharmacological agents on the nasal volume. In order to test the adequacy of our experimental technique, and in order to facilitate the interpretation of our results, we have compared the effects of amyl nitrite and epinephrine on the nasal volume (and nasal temperature) of our subjects with the effects described by Jackson. Figure 2 shows the effect of oral inhalation of amyl nitrite. In this experiment the body of the subject had been exposed to a cold environment

(12.5°C.) for about 27 minutes before administration of the drug. The result for the nasal volume is in agreement with that of Jackson. (Jackson used sodium nitrite.) Such a result, involving elevated temperature of the mucosa and diminished volume (i.e., swelling of the mucosa) of the nasal chamber, we shall designate *hyperemia.*

The action of epinephrine (0.75 cc. of 1:1000 injected subcutaneously) is the opposite of that of amyl nitrite, involving a pronounced drop in nasal temperature and increase in volume (i.e., shrinkage of the mucosa) of the nasal chamber. This result is again in accord with Jackson's findings for the effect of epinephrine on the nasal volume. Such a result we shall designate *ischemia.*

The after-effects of amyl nitrite and epinephrine are worthy of mention. With the first, a reactive shrinkage of the nasal mucosa occurs; with the second, a reactive swelling.

C. *The effect of thermal stimulation upon the nasal temperature and volume.* The most common type of response of the nasal mucosa to *general* cutaneous chilling is shown in figure 3. The body of the subject was exposed for 24 minutes to an environment at 12°C. Following a sudden initial drop in nasal temperature and increase in nasal volume (ischemia), there occurred a gradual decrease in the nasal volume while the temperature of the mucosa was still dropping. This type of response we shall designate *turgescence,* without attempting to specify whether the decrease in volume is upon the basis of vascular stasis, edema or a combination of the two. Our method cannot distinguish between these possibilities.

Unlike the results of local chilling, the finger temperature in such an experiment continues to drop, characteristically approaching room temperature as an asymptote.

Occasionally we have observed a most interesting type of response when the body of the subject was warmed after prolonged exposure to a cold environment. This response involves a rise in nasal temperature, accompanied by an *increase* in nasal volume. This type of response we shall designate *release of turgescence.*

In order to discover whether or not there is a physiological basis for the belief expressed by some otorhinolaryngologists that a cold draft on a warm subject breathing warm air causes a shift of blood to the nose, resulting in "congestion," we studied the effects of a cold foot bath (13°C.) upon subjects exposed to a high room temperature (29°C.). Following a pronounced drop in nasal temperature, associated with a pronounced increase in nasal volume (similar to the effects of epinephrine), there occurred a very rapid recovery, to a point far beyond the initial levels, while the stimulus was still being applied. Our experiments therefore support the opinion of the otorhinolaryngologists. In this case we would prefer to use the term "hyperemia" rather than "congestion."

Discussion. I. The four possible combinations of changes in temperature and volume noted in the nose, namely: 1, rise in temperature and swelling of the mucosa (hyperemia); 2, drop in temperature and shrinkage of mucosa (ischemia); 3, drop in temperature and swelling of mucosa (turgescence); 4, rise in temperature and shrinkage of mucosa (release of turgescence), may be easily

duplicated experimentally in other parts of the body. Types 1 and 2 above scarcely need further comment. Type 3 response may be duplicated in the finger tip, for example, by obstructing the venous outflow, and type 4 by then releasing the obstruction.

It is not surprising, considering the known erectile structure of the nasal mucosa (especially that of the inferior turbinate), that responses like those of types 3 and 4 should be observed. One cannot, simply on the basis of a drop in temperature, conclude that the mucosa may not at the same time be in a turgid state, nor does *blanching* of the mucosa necessarily mean *shrinkage*, since there may still remain a turgescence due to edema, as was shown many years ago by Hill and Muecke (9). It was on the basis of these criteria (drop in temperature and blanching) that Mudd et al. (ibid.) believed that they had invalidated the notion that chilling may cause turgescence of the nasal mucosa.

II. With *local* cutaneous chilling, our results for the nasal temperature responses of normal subjects are in substantial agreement with those of Spiesman, and Spiesman and Arnold (ibid.). We have rarely seen the type of response described by Spiesman after local warming of normal subjects. Spiesman claimed that the nasal temperature, after rising, tended to drop toward the original level within a period of about 10 minutes, while we have found that the nasal temperature nearly always remains elevated so long as the hot stimulus is being applied. (See fig. 1 for a typical example.) In the case of *general* cutaneous chilling and warming, Spiesman's results are in gross variance with ours, since we have found that the nasal temperature tends permanently to fall or rise, respectively, so long as the stimulus is being applied.

Contrary to the findings of Spiesman and Arnold, we have been unable to find any systematic differences between normal and hypersensitive subjects as regards changes in nasal temperature in response to cold and hot stimuli. In three experiments on one of our allergic subjects we did observe a rise in nasal temperature in response to local cold stimulation, and in one experiment a drop in nasal temperature in response to a local hot stimulus. This particular patient, however, was of such an apprehensive and emotional temperament that we hesitate to specifically ascribe the results to the allergic state. In a personal communication to us, Spiesman said, "The allergic patient is not constantly hypersensitive. There are hours in each day and there are often many days during which a hypersensitive person will react as the normal. It is only during the hypersensitive phase that he provides the abnormal response to a cold stimulus. Frequent examinations disclose this fact and are therefore necessary." We are not in a position either to affirm or deny this statement.

The nasal temperatures recorded by Spiesman and Arnold appear to have averaged much higher than ours, which initially nearly always lay between 31° and 35°C. at ordinary room temperature. Our figures agree well with the findings of Mudd et al. (ibid.). We suggest that a possible, and perhaps important, source of error in the experiments of Spiesman and Arnold lay in the size of their thermocouple, which was about 4 mm. in diameter. Particularly in the case of turgescence, spuriously high temperatures might have been recorded.

SUMMARY

1. Nasal temperature and volume changes in response to cutaneous chilling and warming have been studied in a series of normal, common-cold susceptible and hypersensitive human subjects.

2. There is a rough parallelism between changes in nasal and finger temperatures.

3. Local chilling characteristically results in a drop of nasal temperature, with substantial return toward the initial level within a few minutes. Local warming, after a transitory reflex drop, is generally followed by a permanently elevated nasal temperature so long as the stimulus is being applied. No systematic differences between normal and hypersensitive subjects have been observed.

4. Local chilling of a subject exposed to a high environmental temperature results in a pronounced nasal hyperemia.

5. With general cutaneous chilling and warming, four types of nasal response have been observed, designated hyperemia, ischemia, turgescence and release of turgescence. These responses, involving changes in both temperature and volume of the nasal mucosa, are readily duplicated elsewhere in the body.

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REGULATION OF THE RELEASE OF PITUITRIN BY CHANGES IN THE OSMOTIC PRESSURE OF THE PLASMA

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The neurohypophysis receives a generous nerve supply from the hypothalamus and there is considerable evidence that this nervous pathway is the efferent limb of a reflex arc which can be activated by stimulation of somatic afferents. For example, painful cutaneous stimulation will inhibit a water diuresis in the dog by releasing pituitrin from the posterior lobe of the hypophysis (10, 11). Haterius (5) has also shown that mechanical stimulation of the lumbar vertebrae of the rabbit will interrupt the normal course of a water diuresis and that the response is abolished by transecting the hypothalamico-hypophysial tract. There is additional evidence that changes in the composition of plasma will modify the release of pituitrin. The injection of hypertonic NaCl solutions (1, 3, 8) or dehydration by restriction of fluid intake (1, 2, 6, 8) increases the rate of pituitrin liberation, in some cases as much as a hundred fold. The mechanism through which dehydration or hypertonic salt solutions act to release the antidiuretic hormone from the neurohypophysis has not been identified. As a preliminary to that investigation, the present report deals with changes in the plasma which are consistently associated with changes in rate at which pituitrin is discharged into the blood stream.

During dehydration or during the infusion of hypertonic NaCl solutions the total osmotic pressure and the chloride and sodium content of the plasma are all elevated. It is not clear which of these is the effective stimulus for the release of pituitrin. In the present experiments, by injecting solutions of Na_2SO_4, urea and NaCl, we have been able to vary the three factors independently.

It was difficult to formulate an expression generally applicable to the renal responses to these different solutions which indicated the quantity of pituitrin acting upon the kidneys. In earlier experiments in which NaCl was injected, the release of pituitrin was evidenced by changes in the ratio of chloride concentrations of the tubular reabsorbate and of plasma, the chloride R/P. This ratio is without significance, so far as the action of pituitrin is concerned when solutions of Na_2SO_4 or urea are injected. We therefore resorted to other procedures for determining whether the release of pituitrin was increased or decreased by the injection of a given solution.

The most satisfactory of these is that of adequate replacement therapy. There is a marked difference between the responses of normal and chronically polyuric dogs to the infusion of hypertonic salt solutions and this difference can be diminished or almost eradicated by adding graded doses of pituitrin to the fluid infused into the polyuric dog (3). This method is therefore not only qualitative; i.e.,

it indicates a change in the rate of pituitrin secretion into the recipient's blood, but it is also reasonably quantitative. It is assumed that the amount of pituitrin required to convert the response of the polyuric dog to normal is the quantity secreted by the normal dog in response to the infusion. Since this amount has, in a few experiments, exceeded 1500 milliunits it was anticipated that an antidiuretic assay of the normal dog's blood collected before and after the infusion would reveal a measurable increase in circulating pituitrin. Even though the assay method will reveal the presence of 1.0 milliunit per 100 cc. of blood (4), the blood assays have been consistently negative.

The third procedure, the antidiuretic assay of urine collected before and after the infusion, was successful in a large majority of cases.

The fourth was, in principle, a modification of the second. The blood was assayed, not by its antidiuretic effect when transferred into a dog with diabetes insipidus, but by its action on the kidneys of the dog receiving the injection. This was accomplished by making the injection during a sustained water diuresis in a normal dog. A temporary depression in the water diuresis was considered evidence of the release of pituitrin. This technique has been used by Hickey and Hare (7) as the basis of a diagnostic test for differentiating diabetes insipidus due to neurohypophysial damage from the polyuria caused by excessive water drinking. In performance, it is the simplest of the procedures.

Not all of these tests were used in evaluating each solution as a stimulant to pituitrin secretion. When a decisive answer was obtained by two of the methods, it was usually accepted as final. In a few instances, when the results were equivocal, all four procedures were employed.

Procedures. In earlier publications we have given the details of the collection and analyses of plasma and urine (3) and the technique of antidiuretic assay of blood and urine (4). Osmotic pressures were not determined directly but were calculated from chemical analyses of plasma and urine. These analyses always included Cl, Na and urea.

All observations were made on female dogs trained to lie quietly without restraint and accustomed to catheterization and venipuncture. All infusions were given at the rate of 10 cc. per minute for 45 minutes.

Results. *Replacement therapy.* All the solutions, when infused into normal dogs, caused a profuse diuresis. When hypotonic solutions were used, the diuresis, readily suppressed by the addition of small doses of pituitrin (5–25 mu./hr.) to the infusion (fig. 1), was attributed to a diminution in the normal rate of pituitrin secretion. When hypertonic solutions were infused, the addition of 25–250 mu./hr. did not affect the rate of urine flow (fig. 1).

The infusion of 2.5 per cent NaCl solution produced a much greater diuresis in a dog with diabetes insipidus than in a normal dog of the same weight (compare bottom and top curves of fig. 2). The response of the polyuric dog was progressively altered toward the normal by the addition of pituitrin in amounts that provided infusion rates of 60, 180 and 720 mu./hr., but even the largest dose was far short of complete replacement.

A similar experiment with 3.9 per cent Na_2SO_4 (fig. 2) shows that far more than 300 mu./hr. is required to restore the response to normal.

A 6.3 per cent urea solution produced a marked increase in urine flow in the normal dog, but the addition of pituitrin caused such an antidiuresis that it is apparent that this concentration of urea did not elicit the release of a supramaximal amount of pituitrin (fig. 3). The concentration was increased three-fold and the experiment repeated (fig. 3). While the urine flow was increased further, the addition of pituitrin was without effect. The infusion of the 18.9

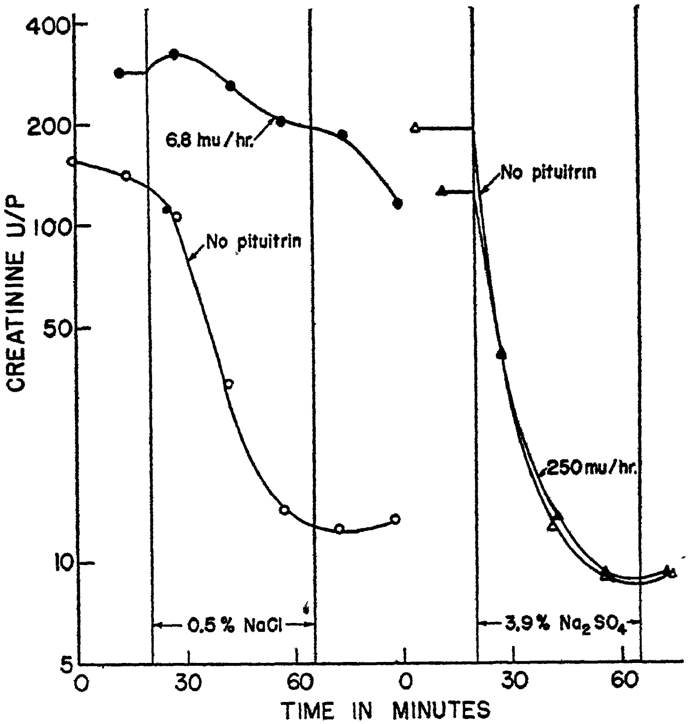

Fig. 1. A comparison of the effects of pituitrin on the diuresis caused by the infusion of hypotonic (left) and hypertonic (right) solutions into the same normal dog. The vertical lines indicate the period of infusion.

per cent urea solution into a dog with diabetes insipidus forced the creatinine U/P below 3.0. The dose of pituitrin added (250 mu./hr.) was not adequate to erase the difference between the responses of the polyuric and normal dog (fig. 3).

The use of creatinine U/P ratios as the index of pituitrin activity has imposed a stringent requirement upon the data on replacement therapy. Since the rate of urine flow is greatly dependent upon the excretion rate of the injected solute, comparisons can be made of only those experiments in which these rates are practically identical. The infusion, at a constant rate, of the same volume of the

same solution into the same dog on different occasions will, in most cases, produce almost identical excretion curves (3). But when different dogs receive the same infusion, the excretion rates differ, in some cases, by as much as 50 per cent and this difference is reflected in the creatinine U/P. We were, therefore, forced to select only those experiments in which the two dogs, one normal and the other polyuric, had the same rate of glomerular filtration and excreted the injected solute at the same rate.

Antidiuretic assay of blood. In this assay, 40 to 50 cc. of blood is transfused, before and after the 45-minute infusion, from the normal into a polyuric dog.

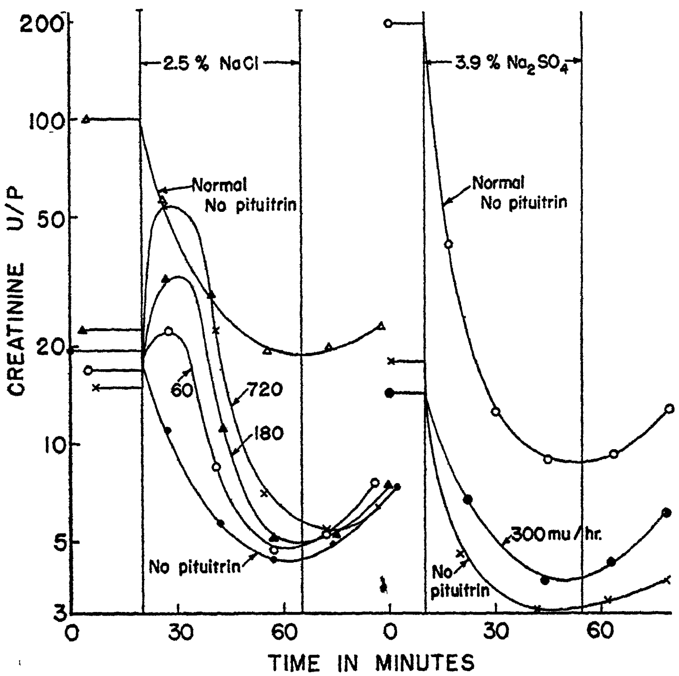

Fig. 2. Effects of replacement therapy with pituitrin in a dog without a neurohypophysis. On the left are the responses to 2.5 per cent NaCl; on the right, to 3.9 per cent Na_2SO_4. The milliunits of pituitrin per hour are indicated by the numbers at the ends of the arrows. The response of a normal dog is included for comparison.

An increase in the creatinine U/P of the recipient dog is evidence of pituitrin activity and by this method concentrations as low as 1.0 mu./100 cc. of blood can be detected. The information obtained through replacement therapy indicates the release of more than 720 mu. of pituitrin per hour in response to the intravenous infusion of 450 cc. of 2.5 per cent NaCl into a normal dog weighing 15 kgm. If as much as 10 per cent of the body weight was blood, this amount of pituitrin should add, during the course of the infusion, about 40 mu. to each 100 cc. of blood. While blood rapidly inactivates pituitrin, the survival of 3 per cent of the active hormone would leave the concentration within the range of the assay method. The only assay suggesting a positive result is that shown in

figure 4, and the increase in the creatinine U/P is too small to be significant. All other blood assays have been clearly negative.

Antidiuretic assay of urine. A positive assay of urine is shown in figure 4. These assays are so well controlled that a positive result is highly significant. In the first place the responsiveness of the assay dog to a standard dose of pituitrin is checked before and after the urine injections. Secondly, the negative assay of the urine collected before the infusion excludes the possibility of the dog's urine

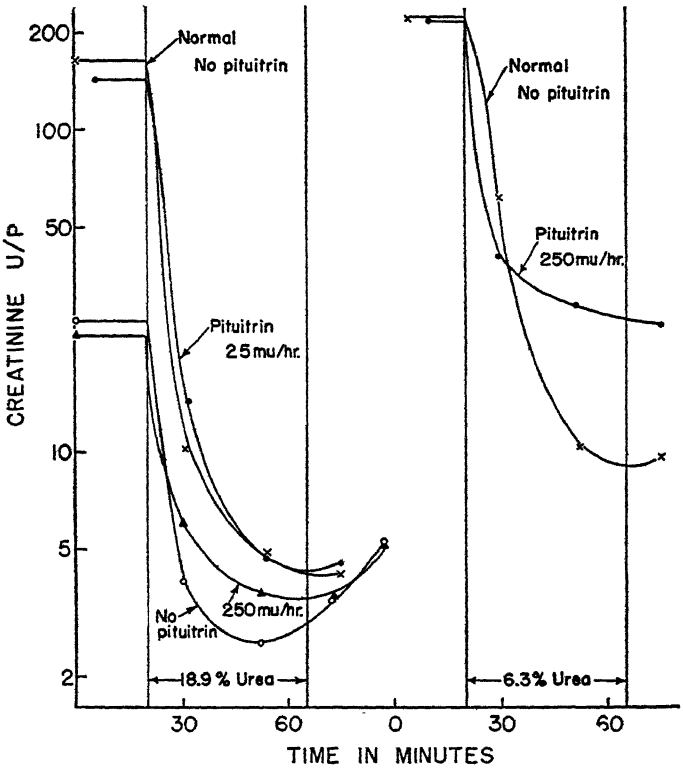

Fig. 3. The effect of pituitrin on urea diureses. The two top curves on the left (crosses and solid circles) show the response of the same normal dog to the infusion of 18.9 per cent urea with and without added pituitrin. The lowest curve (open circles) is the response of a dog with diabetes insipidus. The curve through the solid triangles shows the effect of adding pituitrin at the rate of 250 milliunits per hour. Both curves on the right are based on results from a normal dog. Since pituitrin inhibited the diuresis, it is concluded that the infusion of 6.3 per cent urea does not cause the release of a supramaximal dose of pituitrin.

per se increasing the renal tubular reabsorption of water. The positive assay of the urine collected after the infusion can be attributed, therefore, to the renal excretion of an antidiuretic substance in response to the administration of hypertonic salt. Negative assays are not so significant, as the first urine injection may initiate, in the assay dog, a diuresis not susceptible to control by small doses of pituitrin. Positive assays of urine have been obtained following the infusion of 2.5 and 3.0 per cent NaCl and of 3.9 per cent Na_2SO_4.

Inhibition of water diuresis. Normal dogs with a water diuresis respond to the injection of a few milliunits of pituitrin by a temporary decrease in the rate of urine flow. We have shown that the injection of hypertonic solutions will similarly depress a water diuresis in normal dogs, but augment urine secretion in a dog with experimental diabetes insipidus. As a rule, observations were made simultaneously on four dogs, two normal and two with diabetes insipidus. Five hundred cubic centimeters of water were given by stomach tube to each dog one hour before the observations were begun; 250 cc. were given at hourly intervals thereafter. Urines were collected through retention catheters at 5–10 minute intervals into graduated cylinders. Injections of 25 to 50 cc. of the solution being studied were made intravenously after preliminary collections of 2 or 3 urines to establish the rate of urine flow. One of these experiments is shown in figure 5. The difference between the urinary response of the normal

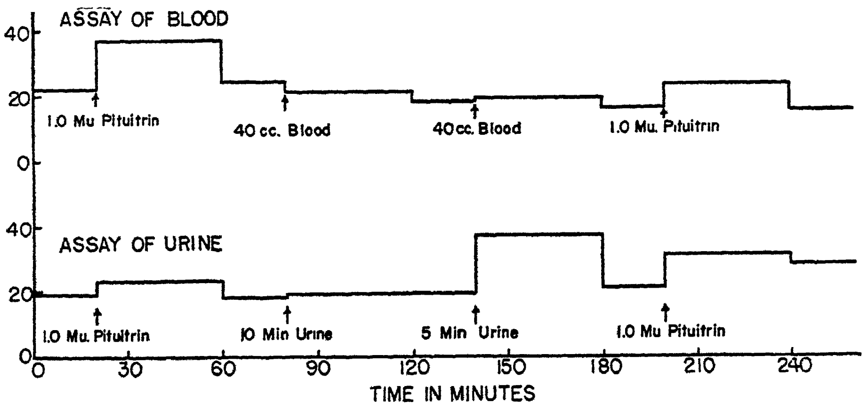

Fig. 4. Antidiuretic assays of blood and urine collected from a normal dog before and after an infusion of 2.5 per cent NaCl. The urine collected after the salt administration produced a strongly positive response, but the blood caused such slight elevation in the urine concentration that its antidiuretic content is questionable. Creatinine U/P is plotted on the ordinate.

and the polyuric dogs reflects the difference in the quantities of pituitrin in their circulation. The normal pituitary releases sufficient pituitrin to cause an antidiuresis; the polyuric dog, with an atrophic neurohypophysis, cannot respond to the stimulus. These polyuric dogs served as controls for all injections and their diuretic responses eliminated the possibility that the antidiuresis of the normals was caused by any action other than the secretion of pituitrin. NaCl, in 5, 10 and 12 per cent solutions, and urea in 12.5 and 25 per cent solutions have caused severe diuresis in the dogs with diabetes insipidus and antidiuresis in the normal dogs.

When it had been established by the preceding methods that concentrated solutions of NaCl, Na_2SO_4 and urea increased the secretion of pituitrin and that dilute solutions had an inhibitory action, we reviewed the data in an attempt to find some aspect of renal activity which would reveal changes in pituitrin secre-

tion regardless of the solute injected. It was clear by this time that changes in the total osmotic pressure of the plasma, rather than changes in any specific ingredient, determine the response of the neurohypophysis. However, the osmotic U/P ratios which offer the great advantage that they can be calculated from direct determinations, do not indicate, even qualitatively, changes in the rate of pituitrin secretion. This is shown in figure 6 in the case of the urea infusions; both 6.3 and 18.9 per cent urea infusions caused a profound fall in the osmotic U/P. McCance and Young (9), who followed the osmotic changes in plasma and urine following restricted fluid intake and after the administration of hyperosmotic solutions of salt and urea, made the same observation. It is sur-

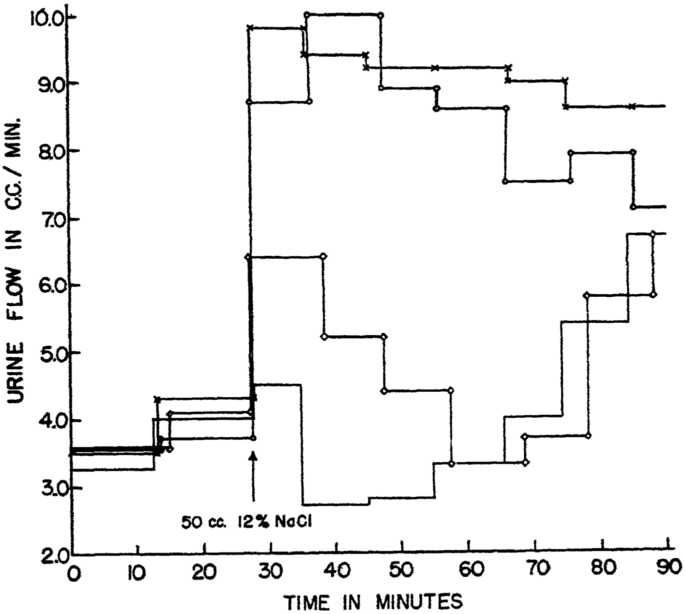

Fig. 5. The urinary response of two normal and two polyuric dogs to the intravenous injection of 50 cc. of 12 per cent NaCl. Crosses and open circles represent dogs with experimental diabetes insipidus; the lower two lines, normal dogs.

prising that a gravely dehydrated subject, on receiving a solution of high osmotic pressure will respond by excreting a less concentrated urine. However, the course of the osmotic R/P did signify changes in pituitrin secretion. The value of R, which can be derived only by calculation as the tubular reabsorbate is not accessible to examination, is obtained from the formula

$$R = \frac{(P)(GF) - UV}{GF - V}$$

where P = os-millimols /cc. in plasma
U = os-millimols /cc. in urine
V = urine flow in cc./min.
GF = glomerular filtration in cc./min.

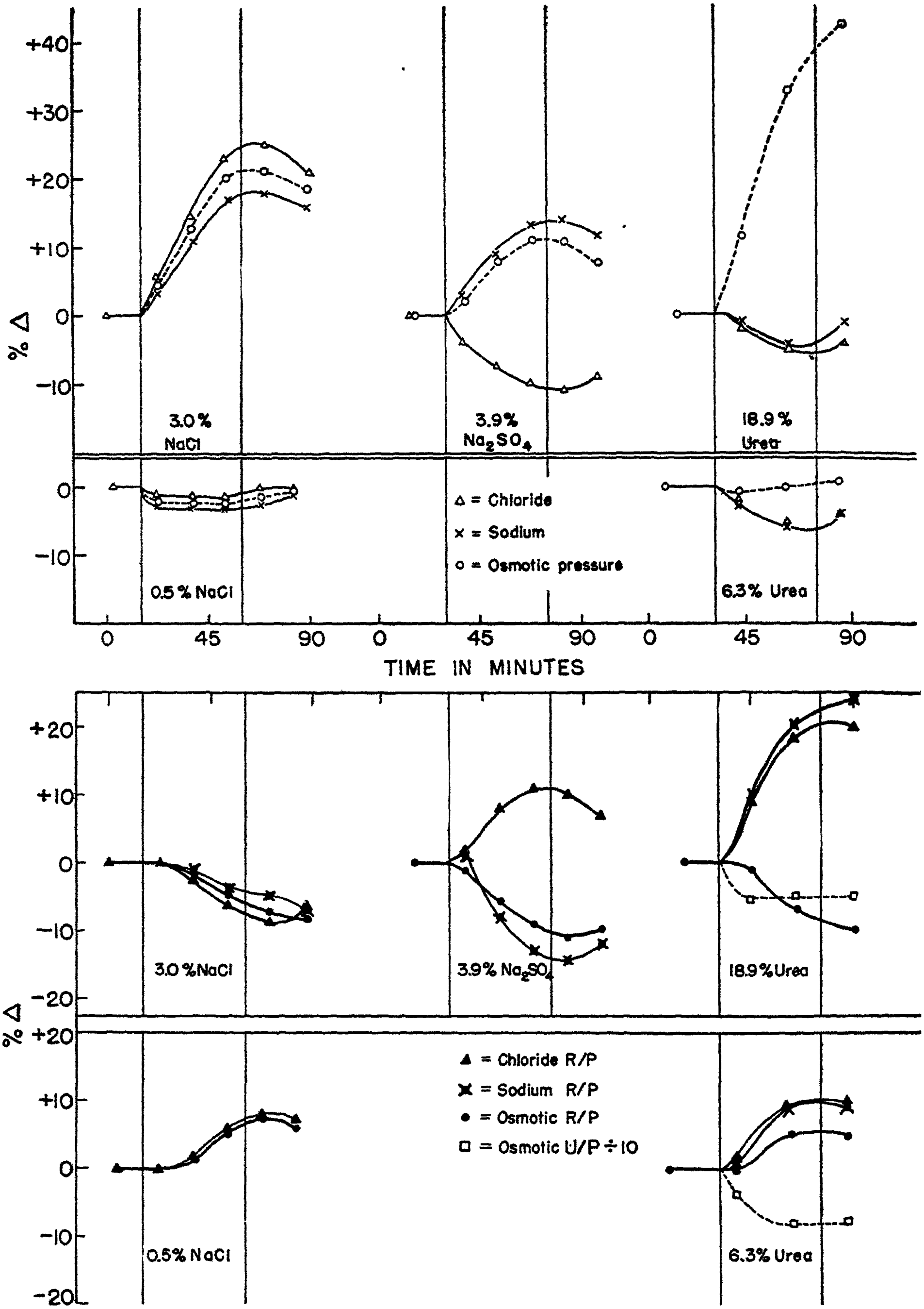

Fig. 6. Changes in the character of the plasma (upper half), tubular reabsorbate and urine (lower half) induced by infusion of five different solutions into the same normal dog. The abscissa represents time in minutes; the ordinate, percentile change from the original value. The percentile change in the osmotic U/P is reduced to one-tenth for accommodation to this scale. The vertical lines enclose the periods of infusion.

When an infusion lowered the osmotic pressure of the plasma, the osmotic R/P was elevated above its normal value of one; when the plasma osmotic pressure was elevated the osmotic R/P was lowered. As might be expected, the osmotic pressure of the infusion fluid provided little basis for predicting the change which would be caused in the plasma. The distribution within the body fluids and the rate of excretion determined to a large extent the change produced in the plasma. While 3.0 per cent NaCl is practically isosmotic with 6.3 per cent urea, the former causes a rise and the latter a slight fall in the osmotic pressure of the plasma when infused intravenously under the conditions of our experiments (fig. 6). It is surprisingly difficult to lower the plasma osmotic pressure by infusing hypotonic solutions. A copious flow of extremely dilute urine results in the retention of practically all the solute and little of the water injected. The plasma chloride has actually increased during the infusion of a salt solution with a chloride concentration lower than that of the plasma. On the other hand, the osmotic pressure of the plasma can be greatly increased by the administration of more concentrated solutions.

Three relationships between plasma and tubular reabsorbate were calculated; the chloride R/P, sodium R/P and osmotic R/P. The last of these is the only one invariably related to changes in pituitrin secretion. Reference to figure 6 reveals that infusions which reduced the osmotic pressure of the plasma caused an increase in the osmotic R/P. The diuresis in these cases (0.5 per cent NaCl and 6.3 per cent urea) was suppressed by pituitrin (figs. 1 and 2). Three per cent NaCl, 3.9 per cent Na_2SO_4 and 18.9 per cent urea solutions all elevated the osmotic pressure of the plasma, lowered the osmotic R/P and caused a diuresis completely refractory to pituitrin.

Discussion. In an earlier paper we proposed the chloride R/P as an index of the quantity of pituitrin released into the circulation in response to the injection of NaCl solutions. While this conclusion is still valid in the light of these more extensive experiments, it is also obvious that the chloride R/P is limited in its applicability to experiments where only NaCl infusions are involved. When other solutes are used it is apparent (fig. 6) that the osmotic R/P is the significant ratio. When the experiments are compared on this basis, it is clear that the osmotic R/P reflects the stimulation or inhibition of the neurohypophysis.

CONCLUSIONS

The intravenous administration of solutions which elevate the osmotic pressure of the plasma cause an increase in the secretion of pituitrin. The renal tubules respond to this increased amount of pituitrin in the circulation by reabsorbing from the glomerular filtrate a solution with an osmotic pressure less than that of the plasma. Solutions which lower the osmotic pressure of the plasma produce the opposite effect in that they inhibit the release of pituitrin. This allows the renal tubules to return to the blood a hypertonic solution.

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THE EXCRETION OF CONJUGATED EPINEPHRINE RELATED COMPOUNDS

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It has been known for some time that certain phenols are excreted in urine both as free compounds and conjugated with sulfuric acid. Richter (1) has described a method by means of which he was able to determine that epinephrine, "Epinine" and "Cobefrin" were conjugated in the body and appeared in the urine in rather large amounts in this inactive form. He concluded logically that conjugation of these compounds with sulfuric acid took place through one of the hydroxyl groups on the phenyl nucleus.

It appeared desirable to repeat and extend this past work for a number of reasons. As a principal form of inactivation of these compounds this reaction is not particularly consistent with the general views on the subject. It seemed best to use Richter's general method to determine qualitatively for ourselves whether conjugation and excretion of epinephrine actually occurred. However, the methods reported appeared likely to give but a rough approximation of the amount of these chemicals excreted. Consequently, we have adapted the test to photocolorimetric methods for a more accurate quantitation of our results. Also, we have presented additional experiments which support the evidence for this mode of inactivation.

The purpose of this research, then, has been to determine the significance of conjugation as a mode of elimination of epinephrine and related ortho-dihydric compounds from the body.

Experimental. *Isolation and quantitation.* Our method for the isolation from urine and color formation of the epinephrine derivatives was fundamentally that presented by Richter, except for differences necessitated by our procedure for estimating the concentration of the colored end product. Our method was as follows:

The preparation of urine for hydrolysis differed with the type of experiment performed. This will be explained with the description of the different experiments. Forty cubic centimeters of the prepared urine were refluxed with 3 cc. of concentrated sulfuric acid for one-half hour on a boiling water bath. To duplicate 15 cc. aliquots of the colored hydrolyzed urine were added 0.5 cc. of 1.5 per cent glycine solution, 0.2 cc. of saturated sodium acetate solution and sufficient thymol blue to color the urine distinctly. This urine was adjusted rapidly to pH 5.0, using a Gilson photoelectric pH apparatus, and immediately

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filtered into 50 cc. centrifuge tubes. For accurate determinations the pH of the solution should not be allowed to exceed 6 at this point, since readjustment with acetic acid does not give very satisfactory results. The material on the filter was washed with about 10 cc. of water. Two cubic centimeters of an aluminum hydroxide suspension[2] were added to each tube and the contents brought to a pale grey color by the drop-by-drop addition of 10 per cent sodium hydroxide solution. Excessive addition of base was avoided. The suspension was shaken for 5 minutes, centrifuged and the supernatant liquor poured off. The centrifugate was resuspended by adding to it 1 cc. of 25 per cent sodium dihydrogen phosphate solution and 6 cc. of water. After the resuspended material had stood for 5 minutes it was centrifuged again. The supernatant solution was then poured into an Evelyn photoelectric colorimeter tube containing 0.4 cc. of 5 molar sodium acetate buffer solution, pH 5.2, and then 0.5 cc. of a 0.1 N solution of iodine in 5 per cent potassium iodide was added. After 5 minutes 0.5 cc. of 0.1 N sodium thiosulfate solution was added rapidly, the tube shaken to mix, and the reading of the maximal galvanometer deflection recorded immediately. The galvanometer center setting should be determined previously, using the reagents and water for the blank, a 520 mμ filter and a 6 aperture.

The concentration of a given compound whose color intensity had been determined in terms of its galvanometer deflection was read from a curve previously established by plotting the color intensity of serial dilutions against the corresponding galvanometer deflection and correcting for urine volume and dilution.

Certain points of difficulty in using this procedure must be recognized and guarded against. One must be careful to conform to the procedure; else interfering substances which are orange in color come through to mar the results. The greatest difficulty is that the colors fade rather rapidly in some instances so that readings must be taken within 30 seconds of the beginning of sodium thiosulfate addition to the solution in the tube. It has been our practice to record the maximal galvanometer deflection (allowing of course for the fling of the galvanometer).

The recoveries of known amounts of the amines added to urine were determined in order to test the accuracy of the procedure. Duplicate 15 cc. aliquots of these preparations were taken through the procedure unhydrolyzed, and the concentration of the amine in the final step measured. Forty cubic centimeters of each of these preparations were hydrolyzed as described and 15 cc. aliquots (plus a correction for the volume of sulfuric acid added) of the hydrolyzed urine were taken through the same analytical scheme. The results of one set of these experiments are recorded in table 1. From table 1 it may be concluded that the recovery of these amines from urine was satisfactory, considering the character of the procedure and the magnitude of the concentrations of the compounds.

Mode of excretion of epinephrine, "Epinine" and "Cobefrin". The dose for

[2] Dissolve 50 grams of potassium alum in 400 cc. of hot water, cool to room temperature and stir in rapidly 10 grams of sodium hydroxide previously dissolved in 40 cc. of water. Filter, wash with water and resuspend the aluminum hydroxide in 200 cc. of water.

oral administration of all the compounds was 30 mgm. of the hydrochloride, except for epinephrine which was used as the base. This was given together with 200 mgm. of glycine and 1 cc. of 5 per cent acetic acid dissolved in 50 cc. of water just before it was consumed. This was washed down with 100 cc. of water.

Just before taking the drug the bladder was emptied—by catheterization in the case of the dogs. For the qualitative determination of excretion of these compounds, urine was collected for only 4 to 6 hours following ingestion of the compound, and 15 cc. aliquots were tested for free and combined epinephrine, etc.

It was found that within the limits of this test none of the compounds was excreted as such. It was also determined that in every instance "Epinine", epinephrine and "Cobefrin" were excreted in a combined form which could be hydrolyzed.

The time of onset and maximal excretion of each of these compounds were determined by collecting urine specimens at 2-hour intervals after a 30 mgm. dose of any one of the compounds was taken orally. In the case of the dogs this necessitated catheterization at the beginning of an experiment to remove the residual urine and again every 2 hours for 4 periods (a total of 8 hr.) thereafter. In a few instances this was carried on for 12 hours. Twice during the 8 hours the dogs were given about 200 cc. of water by stomach tube to insure an adequate urine flow. The volumes for both the human and dog urines for the 2-hour periods were recorded and made up to 100 cc. for the period unless the recorded volume exceeded that figure.

TABLE 1

Recovery of epinephrine, "Epinine" and "Cobefrin" added to urine and the effect of acid hydrolysis of the urine on the recovery

COMPOUND	AMOUNT ADDED TO 15 CC. OF URINE	AMOUNT RECOVERED FROM UNHYDROLYSED URINE	AMOUNT RECOVERED FROM HYDROLYSED URINE
	mgm	*mgm.*	*mgm.*
Epinephrine........	0.02–0.02	0.015–0.015	0.020 –0.025
"Cobefrin"..........	0.02–0.02	0.02 –0.0195	0.0195–0.0195
"Epinine".	0.02–0.02	0.023–0.023	0.025 –0.026

Table 2 summarizes the results of these experiments. It may be seen that in every instance these compounds appeared in the urine in considerable quantities within 2 hours following their ingestion. Maximal excretion of the amines occurred within the first 4 hours following the time they were taken.

The total 24 hour excretion of "Cobefrin" and "Epinine" by man is given in table 3. Surprisingly, on the average more "Epinine" than "Cobefrin" was excreted over this period. It was also of interest that such very great recoveries of the compounds from hydrolyzed urine were possible (83.7 per cent for "Epinine" and 70.4 per cent for "Cobefrin", average).

These data (tables 2 and 3) seem to indicate that most of a given dose of any

of these compounds administered orally was excreted by the kidneys in a conjugated form.

The route of administration of the compounds was considered as possibly influencing our results. The compounds were administered at different times either orally or subcutaneously to dogs. The toxic reactions to 20 or 30 mgm. of either epinephrine or "Cobefrin" were so severe when administered subcutaneously that the results appeared to be influenced appreciably thereby.

TABLE 2

The progressive eight hour recovery from acid-hydrolysed urine of a conjugated form of "Cobefrin", "Epinine" and epinephrine when these compounds were administered orally together with glycine and acetic acid to dogs and humans

SUBJECT	DOSE	URINARY RECOVERY IN MGM.				PERCENTAGE RECOVERED IN 8 HR.
		2 hr.	4 hr.	6 hr.	8 hr.	
"Cobefrin" hydrochloride						
	mgm.					
Dogs						
A	30	3.09	5.35	0.53	0.40	31.2
B	30	1.60	1.83	0.58	0.33	14.4
"Epinine" hydrochloride						
A	30.5	10.50	9.97	2.46	0.99	78.5
B	30.5	7.25	5.32	1.67	0.73	49.2
Epinephrine base						
A	25	2.53	0.93	0.33	0.10	15.9
B	25	1.96	2.80	0.60	0.23	22.3
"Cobefrin" hydrochloride						
Humans						
TL	30	1.15	3.52	2.01	2.40	30.4
DB	30	4.03	2.03	2.12	2.05	34.1
"Epinine" hydrochloride						
JD	30	3.26	9.32	6.13	4.57	77.6
BH	30	5.56	5.98	8.23	3.54	77.8

Since the dogs were upset least by "Epinine" these results were considered the most reliable and have been presented in table 4. Even here, as in the case of the other two compounds, there was less of the material excreted conjugated after parenteral administration than after oral administration. We hasten to acknowledge that the injection of 20 or 30 mgm. of any of these compounds could hardly be considered to similate any physiological state. The purpose of this experiment was to demonstrate, using comparable dosages, that conjugation

of these compounds was not dependent primarily on a single mode of administration.

The effect of liver aminase on epinephrine, "Epinine" and "Cobefrin". Guinea-pig liver was homogenized with an equal weight-volume of distilled water.

TABLE 3

The total twenty-four hour recovery from acid-hydrolysed urine of a conjugated form of "Epinine" and "Cobefrin" when 30 mgm. doses of the hydrochlorides together with glycine and acetic acid were administered orally to man

SUBJECT	URINE VOLUME	AMOUNT RECOVERED	PERCENTAGE RECOVERED
"Cobefrin" hydrochloride			
	cc.	*mgm.*	
L. S.	1560	20.1	67.2
H. S.	835	16.7	55.6
G. P.	1100	19.8	67.1
F. T.	1530	21 4	71.5
J. A.	640	20.5	68.3
"Epinine" hydrochloride			
A. S.	1903	27.9	93.2
C. O.	1030	24.7	82.3
J. F.	1670	26.7	89.2
B. R.	1550	27.9	93.2
D. S.	1030	25.4	84.8
R. Q.	1410	17.9	59.7

TABLE 4

A comparison of the progressive recovery of a conjugated form of "Epinine" from acid hydrolysed urine when the compound was administered orally or subcutaneously to dogs

DOG	DOSE	URINARY RECOVERY IN MGM.				PERCENTAGE RECOVERY IN 8 HR.
		2 hr.	4 hr.	6 hr.	8 hr.	
"Epinine" hydrochloride administered orally						
	mgm.					
A	30.5	10.50	9.97	2.46	0.99	78.5
B	30.5	7.25	5.32	1.67	0.73	49.2
"Epinine" hydrochloride administered subcutaneously						
A	30.0	5 10	3.32	2.72	1.06	40.7
B	30.0	3.32	4.46	6.12	1.03	50.5

Another volume of M/8 sodium-potassium phosphate buffer, pH 7.3, was added, and the suspension strained through muslin. The Warburg apparatus was used. The temperature of the bath was 38.4°C. and the apparatus was adjusted to shake at a stroke of 4 cm. and at a rate of 100 per minute. Filter paper saturated

with 10 per cent potassium hydroxide solution was contained in the center wells. The experiments were set up as follows:

1.7 cc. homogenized liver preparation (fresh)
0.1 cc. M/25 NaCN (fresh)
0.2 cc. M/16 substrate

2.0 cc. total volume

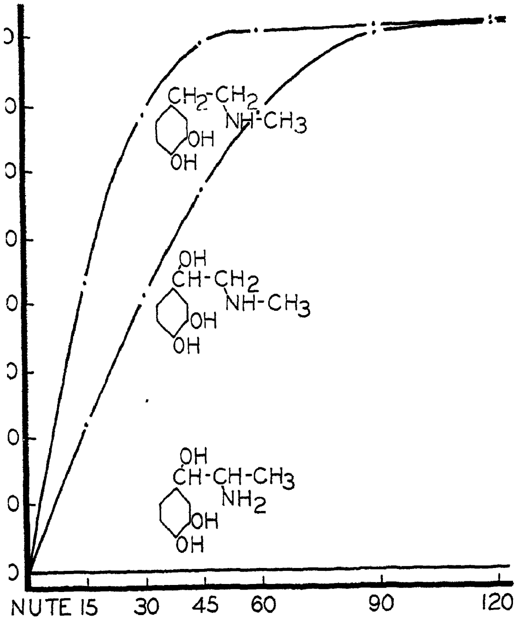

Fig. 1

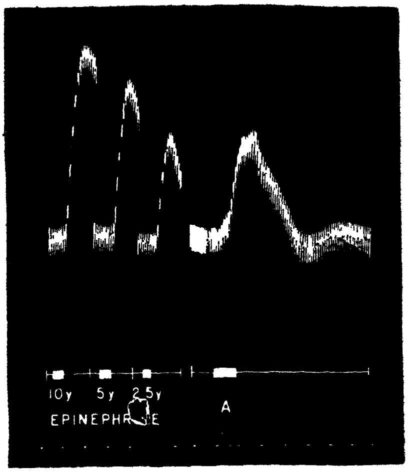

Fig. 2

Fig. 1. Illustrating the relationship between structure and the rate of oxidative deamination of "Epinine" (top curve), epinephrine (middle curve) and "Cobefrin" (bottom curve) by aminase. The compounds were equimolar and the results represent a single typical experiment.

Fig. 2. Illustrating the pressor response in a 10.4 kgm. dog to the intravenous injection of 2.5 to 10.0 γ of epinephrine and to 1.3 cc. of the final extraction material (A) from hydrolyzed urine obtained from a dog which had been given 30 mgm. of epinephrine plus glycine by stomach tube 6 hours previous to catheterization.

The cyanide was necessary to prevent autoxidation of the substrates and also to eliminate respiration of cyanide-sensitive systems which might be influenced by the substrates.

Figure 1 illustrates the results of one of these experiments. Epinephrine and "Epinine" were rapidly oxidized (deaminated) whereas "Cobefrin" was not deaminated by the cyanide-insensitive aminase of the liver. The oxygen uptakes accompanying "Epinine" and epinphrine deamination were slightly in excess of one atom of oxygen per molecule of substrate, probably due to some unavoidable autoxidation of the catechol nucleus. The finding that "Cobefrin" was

not deaminated is consistent with what has been reported previously—that the isopropylamine side chain is not deaminated in the presence of aminase (2, 3).

The relation of molecular configuration of this group of compounds to their deamination by aminase has been studied in considerable detail (4). The importance of these observations with respect to the present study is the fact that epinephrine and "Epinine" as such are rapidly deaminated by aminase whereas "Cobefrin" is not. Yet all three of these compounds are almost entirely excreted in a conjugated form—"Epinine" to the greatest extent.

The effect of phenolase on epinephrine, "Epinine" and "Cobefrin" was considered in detail in a recent report (5). The rapid rate of oxidation of these compounds in the presence of potato phenolase is that of a first order reaction in every instance. It is important to point out that a certain reservation is permissible in believing that a mono- or polyphenolase system exists in normal mammalian tissue capable of oxidizing such compounds as tyramine to 3,4-dihydroxytyramine or the latter compound to the corresponding ortho-quinone in the course of melanin formation. Block (6) and a number of workers since have claimed to demonstrate the presence of such enzymes in mammalian tissues, especially the pigmented layer of the skin. The presence of such enzymes has been questioned byBhagvat and Richter (7). However, two recent reports on the subject warrant consideration and substantiation. Cadden and Dill (8) reported the presence of a polyphenoloxidase in cell-free kidney extracts. This substance oxidized catechol and hydroquinone but not p-cresol, dihydroxyphenylalanine (dopa) or tyrosine, and so was judged by them not to be a tyrosinase but instead to be similar to a laccase (9). Hageboom and Adams (10) have reported the identification of a melanoma tyrosinase and dopa oxidase in preparations derived from a melanoma of mouse origin. Actually, the dopa oxidase very slowly oxidized epinephrine but both oxidases apparently had much more limited specificities than phenol oxidases from other sources. Final judgment of the physiological significance of this observation together with its transposition to normal tissues and other phenolic amines should await the further study that this finding deserves.

Keilin and Mann (11) showed that reduced cytochrome C could oxidize epinephrine *in vitro*. The *in vivo* significance of their observation is questionable. Hydroquinone, catechol and homogentistic acid, which also are oxidized readily by cytochrome *in vitro*, escape this oxidation *in vivo* and appear in the urine as such or in a conjugated form.

The specificity of the test for these compounds must be established before arriving at a definite conclusion from this work. Richter and Blaschko (12) have shown that the color formation in this reaction is due to the formation of an iodoadrenochrome and so is very specific for compounds having a catechol nucleus with an amino group in the beta position on the side chain.

Even so, one can conceive of "Paredrine"[3] (p-hydroxy phenylisopropylamine)

[3] "Epinine" is the trade mark applied by The Burroughs Wellcome Co. to β(3,4-dihydroxyphenyl) ethylmethylamine.

"Cobefrin" is the trade mark applied by Winthrop and Co. to $\beta\beta$(3,4-dihydroxyphenyl) hydroxyisopropylamine.

"Paredrine" is the trade mark applied by Smith, Kline and French Laboratories to

or tyramine (p-hydroxyphenylethylamine) being excreted in part in this conjugated form, especially if a phenolase is present in the body. We have shown that the first step in the oxidation of these compounds by phenolase (3), or the ascorbic acid—dehydroascorbic acid system (13) for that matter, is to the ortho-dihydric or catechol nucleus stage. Should this step occur in the mammalian body, the ingestion of 20 or 30 mgm. of either tyramine or "Paredrine" would enable one to observe much the same color as that observed with epinephrine when the test was applied to urine excreted in the following few hours if it were hydrolyzed and taken through this procedure.

We have taken doses of 30 mgm. of "Paredrine" and tyramine hydrochlorides on different days and have collected urine for 6 hours following ingestion of a given drug. This urine was immediately hydrolyzed and taken through the procedure as usual. We have never obtained a trace of color which would indicate that oxidation of the mono- to the ortho-dihydric nucleus occurred in the body under these circumstances in sufficient quantities to be detectable by present methods.

The biological characterization of the excretion product following the administration of epinephrine or one of the related dihydroxy compounds has been done in the following manner. The compound, epinephrine in the experiment leading to figure 2, was administred to dogs in a dosage of 30 mgm. orally. The bladder previously was emptied by catheterization. About four hours after the dose was administered urine was collected and an aliquot was hydrolyzed and taken through the extraction procedure to the point of addition of the iodine solution. This solution then was injected intravenously into anesthetized dogs. The effect of the urine extract on the carotid arterial pressure was recorded by means of a mercury manometer. Figure 2 illustrates an almost typical epinephrine blood pressure response to the injection.

DISCUSSION. These experiments substantiate the conclusion that epinephrine, "Epinine" and "Cobefrin" were conjugated in the body and excreted in this form. Let us review existing evidence in support of this view.

Epinephrine, "Cobefrin" and "Epinine" were excreted in a form which on hydrolysis yielded the original compounds as indicated by tests of pressor activity and by the test which is specific for those compounds having a catechol nucleus and an amino group on the carbon atom beta to the ring.

Phenol and catechol are conjugated by sulfonation in the mammalian body and are excreted partially in this form. Because of the similar ring structures it might be reasoned that the compounds related to epinephrine should be inactivated similarly. The aliphatic hydroxyl group alpha to the ring might serve to impede but not to inhibit entirely this conjugation, as we have shown to be true for other reactions involving the phenolic nucleus (5).

Unless some change in the molecule took place, "Epinine" and epinephrine would be quickly deaminated by the aminase of the liver. Figure 2 illustrates that fact. Actually, "Epinine" was excreted combined to a greater extent than was "Cobefrin", which was not deaminated by aminase. In all probability this change occurs on the catechol nucleus, for β-phenylisopropylamine is excreted as

such whereas β-phenylpropylamine is not (14). In this group of compounds having no phenolic groups, the position of the amino group with respect to the terminal carbon atom of the side chain determines whether the compound is excreted or deaminated by aminase.

The change in the catechol nucleus is not due to the action of a phenolase in the tissues because "Cobefrin", as well as the other two compounds, could be oxidized by such a system at least to the corresponding ortho-quinone, if such a system were present functionally in guinea-pig liver. Actually, homogenized guinea-pig liver tends to inhibit autoxidation of the compound (3). Also, if this oxidation did occur it would not be reversible by hydrolysis, and one would not get a postive test for these compounds in hydrolyzed urine.

It is possible that the conjugation is of the nature of esterification with sulfuric acid of one or both of the phenolic hydroxyl groups as occurs in the case of phenols. Loeper, Loeper, Lemaire, Cottet and Parrod (15) reported that when tyramine is conjugated through its phenolic group with sulfuric acid it loses its pressor activity. Hydrolysis of this conjugated tyramine with hydrochloric acid yielded free tyramine which on neutralization was shown to regain its pressor and hyperglycemic properties. Richter and MacIntosh (16) have shown that conjugated epinephrine recovered from urine is inactive pharmacologically but can be converted to active epinephrine by hydrolysis. Indirect evidence that sulfuric acid is involved in the metabolism of epinephrine comes from the reports of Deichmann (17) and of Torda (18). The former author reported that when epinephrine was administered orally or parenterally to rabbits there was an incommensurate increase in the renal elimination of organic sulfates. Torda demonstrated an increase in the excretion of conjugated phenol when phenol was administered parenterally to cats. She deduced that this finding substantiated the reports of Richter and MacIntosh (16). Unfortunately only the work of Loeper et al. (15) can be considered direct evidence for such a conjugation of phenolic sympathomimetic compounds with sulfuric acid, and their synthesis of tyramine sulfuric ester has not been repeated successfully in these laboratories.

There are several objections to accepting this as a fundamental concept in the inactivation of epinephrine and its related compounds. Probably the chief objection is that esterification is not in itself an oxidative process, as many believe the inactivation of epinephrine to be. Only recently Bain, Gaunt and Suffolk (19) reported that liver slices did not inactivate epinephrine except in the presence of oxygen. Oxygen is needed in two types of oxidation of epinephrine by aminase and phenolase, as we have shown. Written as a simple equation, the conjugation of epinephrine with sulfuric acid does not involve necessarily the use of oxygen. This has been offered as a criticism of the significance of the reaction *in vivo*. However, in a recent communication Arnolt and de Meio (20) reported that the conjugation of phenol in the liver and intestine is an enzymatic process. They concluded from their evidence that the process, which in itself does not require the consumption of oxygen, requires the energy produced by reactions coupled with oxygen consumption. If it be confirmed, this observation should go far to reconcile sulfuric acid conjugation with epinephrine with the oxidative

process long believed to occur. Since conjugation of "Epinine" and epinephrine does occur it would seem that this system to be effective must be much more active than the sluggish aminase system; otherwise, deamination would also be a significant factor in inactivation. The implications of such a system for the initial inactivation of epinephrine are very attractive.

It might be argued that a "sulfosynthase" is present only in the intestine and liver and so conjugates only the epinephrine homologues brought to it by the portal system. This is not necessarily true, since considerable amounts of epinephrine, "Epinine" and "Cobefrin" are excreted conjugated even after subcutaneous injection.

SUMMARY

The experimental evidence reported herein seems to permit the conclusion that epinephrine, "Epinine" and "Cobefrin" are conjugated through a phenolic hydroxyl group and appear in urine in this form. This mode of elimination is essentially independent of the mode of administration of the amines.

We have presented evidence for and against conjugation as an important manner of detoxication of this group of compounds. We are of the opinion that subsequent work will tend to support this as a principal form of initial inactivation of epinephrine.

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DEVELOPMENT OF HYPERTENSION IN EMOTIONAL GRAY NORWAY RATS AFTER AIR BLASTING[1]

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The emotional makeup of an individual and the stresses imposed upon him are believed to play a part in many cases of hypertension in man (1). The relative importance of these two factors—i.e., emotional pattern of an individual contrasted with environmental stress—in the development of hypertension is difficult to assess in man. The present paper deals with this problem in rats.

METHOD. Twenty-three gray Norway rats, bred and raised under uniform conditions, were used. At ages ranging from 119 to 177 days, they were tested for emotionality in Hall's open field situation (2), and an emotionality score obtained for each rat.[2] Blood pressure determinations by the method of Griffith (3) were then made on thirteen of the twenty-three animals. Following these preliminary steps, eleven of the animals were set aside as controls, while the remaining twelve were subjected to the sound of an air blast five minutes a day for five days a week (4). At the end of this period, when each rat had been air blasted a minimum of 167 times, blood pressure determinations were repeated on all of the animals.

RESULTS. Blood pressures over 160 mm. of mercury were considered hypertensive (5), and in all but two cases hypertensive readings were higher than 180 mm. of mercury. The distinction between emotional and non-emotional rats was a matter of judgment, and, as has been suggested (6), animals with scores of 8 to 12 were considered to be highly emotional, 4 to 7, borderline emotional, and 0 to 3, non-emotional. The results are summarized in table 1.

The initial blood pressure values, measured in thirteen of the rats, fell within a normal range. Following the period of auditory stimulation, a permanent hypertension was established in ten of the twelve experimental rats, while blood

[1] This work was aided by a grant from the Samuel S. Fels Fund.

[2] Briefly, Hall's test is as follows: a rat is placed in a large, well-lighted, circular enclosure 8 feet in diameter for two minutes a day on twelve successive days. This strange environment may evoke an emotional response in the rat, including defecation and micturition. A score is obtained by noting the number of days during which the rat either defecates or urinates while in the enclosure. Animals excreting on no or few days of the test are considered to be non-emotional; rats with high scores (maximum, 12) are called emotional.

pressures of all but one of the control animals were normal. The majority of animals, both experimental and control, had high emotionality scores. All of the experimental animals with scores of 8 or more, however, were found to be hypertensive after air blasting, while none of the highly emotional control animals were or became hypertensive. The only air blasted rats that failed to exhibit an increased blood pressure had emotionality scores of 6 and 3. Two other experimental rats with borderline scores (7 and 6) were found to be hypertensive after air blasting.

Discussion. The procedure of air blasting rats has been shown to result frequently in seizures typical of excessive autonomic stimulation (7), and is believed to be psychically disturbing. It has been shown (5) that continual exposure of rats to the air blast will induce a hypertension maintained under ether anesthesia. This hypertension is assumed, therefore, to be permanent in type. In ten of the twelve air blasted animals in this study, such a hypertension

TABLE 1

AIR BLASTED				CONTROL			
Rat	Emotionality score	Blood pressure (initial)	Blood pressure (final)	Rat	Emotionality score	Blood pressure (initial)	Blood pressure (final)
1 M	12		High	1 F	12	Normal	Normal
2 F	12	Normal	High	2 F	12	Normal	Normal
3 F	11	Normal	High	3 F	11	Normal	Normal
4 F	10		High	4 F	11	Normal	Normal
5 M	10	Normal	High	5 F	10	Normal	Normal
6 M	8		High	6 M	8	Normal	Normal
7 F	8		High	7 F	7		High
8 F	8		High	8 F	7	Normal	Normal
9 M	7	Normal	High	9 M	7		Normal
10 M	6		High	10 M	4		Normal
11 F	6	Normal	Normal	11 F	1		Normal
12 F	3	Normal	Normal				

was produced. Although the two experimental rats failing to exhibit hypertension were of low or borderline emotionality, these two instances are insufficient to suggest that a phlegmatic rat will resist the development of hypertension even when exposed to a stimulus that is adequate to produce high blood pressure in emotional animals.

The results indicate that rats with an inherently unstable temperament will not, if undisturbed, become hypertensive; but if subjected over a long period of time to an emotionally upsetting stimulus, they will develop hypertension. Emotional makeup, therefore, seems to occupy the rôle of a predisposing cause, and environmental stress, that of the exciting factor in the development of a neurogenic hypertension in rats.

SUMMARY

1. Twenty-three Wistar gray Norway rats were tested for emotionality by Hall's method. Twelve of the rats were subjected to a minimum of 167 daily

exposures to the sound of an air blast, and eleven were maintained as controls. Blood pressure determinations were taken on rats from both groups before and after the period of air-blasting.

2. Hypertension developed in ten of the twelve air blasted animals, and in one of the control group. All of the emotional air blasted rats developed hypertension while none of the emotional controls did.

3. It is concluded that a psychically disturbing stimulus will produce neurogenic hypertension in an emotional rat; but if undisturbed, such a rat probably will not develop hypertension.

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OXIDATION OF PYRUVATE AND GLUCOSE IN BRAIN SUSPENSIONS FROM ANIMALS SUBJECTED TO IRREVERSIBLE HEMORRHAGIC SHOCK, CARBON MONOXIDE POISONING, OR TEMPORARY ARREST OF THE CIRCULATION—A STUDY OF THE EFFECTS OF ANOXIA

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We have undertaken a study of the respiratory activity of cerebral tissue from animals subjected to various forms of anoxia with the view of obtaining information as to the mechanism of irreversible hemorrhagic shock. In this condition, transfusion of an amount of blood equal to that previously withdrawn or lost fails to produce more than temporary recovery from hypotension. This raises the question whether exposure to prolonged periods of anemic anoxia results in irreparable damage of the circulation in vital organs or interferes with the ability of the cells to utilize the oxygen supplied by the transfused blood.

There are indications that anoxia might impair the oxidative capability of cells. It has been reported by Greig and Govier (1943) and Greig (1944) that the concentration of coenzymes of oxidative systems is diminished in the tissues of dogs subjected to hemorrhagic shock. Schmidt (1928) and Schmidt, Pennes and Kety (1945), working with the dog and monkey, found that prolonged periods of cerebral anemia produce an irreversible reduction in the ability of brain cells to take oxygen out of the blood. Engel, Harrison and Long (1944) observed that when circulation to the rat liver is occluded for a period of one hour, the ability of this organ to remove amino acids from the blood is irreversibly depressed.

Opinions differ as to the respiratory activity of tissue slices from anoxic animals. Beecher and Craig (1943) failed to detect alterations in the aerobic metabolism of cerebral cortex, cardiac muscle, renal cortex, and liver from cats in profound (though possibly not irreversible) hemorrhagic shock, whereas Russel, Long, and Wilhelmi (1944) reported reduced rates of oxygen consumption in livers from rats in hemorrhagic shock. Since in *in vitro* experiments with rat liver one deals mainly with endogenous respiration (oxidation of cellular substrates) it is difficult to distinguish between destruction of respiratory enzymes, loss of coenzymes or mere lack of oxidizable substrates.[1]

Our investigation of the effect of anoxia upon the oxidative capability of cells has been limited to cerebral tissue for the following reasons: 1. Brain appeared

[1] This behavior of livers from rats in shock resembles that found (Rosenthal, 1937) in livers from rats subjected to prolonged periods of fasting.

to be the only organ for which experimental evidence was available of irreversibly disturbed respiratory ability as a consequence of anemic anoxia. 2. Since the oxidative metabolism of excised cerebral tissue is based upon the utilization of known added substrates, interpretation of quantitative and qualitative alterations of the gas exchange is facilitated. Moreover, the opportunity of testing in a simple manner for a critical deficiency of one of the coenzymes is afforded by the circumstance that cerebral tissue, depleted of thiamine (the precursor of co-carboxylase), responds to the addition of this factor with increased utilization of added pyruvate.

Three types of anoxia were studied: 1, the anemic anoxia of irreversible, hypotensive hemorrhagic shock; 2, carbon monoxide hypoxia (as an example of anemic anoxia without material alteration in blood volume) and 3, stagnant anoxia, produced by temporary arrest of the circulation.

Since unselected groups of dogs and cats were used for the experimental series the possibility had to be considered that differences in breed and dietary history could have a sufficiently great influence upon cerebral metabolism that the effect of anoxia might be concealed. To eliminate this source of error a method of internal control was used in which a brain sample from each animal was analyzed 10 to 20 days prior to the exposure to anoxia and a corresponding sample from the opposite hemisphere was studied following the experimentally induced anoxic state.

METHODS. *Production of anoxia by hemorrhagic shock.* Dogs under light surgical anesthesia (25 mgm. nembutal per kgm. intravenously) were bled until the blood pressure had fallen to approximately 30 mm. Hg. The pressure was kept at this level by means of small withdrawals and reinjections of blood, as required. In two of the dogs, after the blood pressure had been maintained at the above level for 2 hours, the amount of blood previously withdrawn was reinjected. Following the spontaneous return of the blood pressure to a low level in approximately 2 hours, biopsies of the brain were made. A third dog was maintained for three hours at an average pressure of 30 mm. Hg and a brain sample was taken directly without reinjection of blood.

Production of anoxia by carbon monoxide. Anesthetized dogs (50 mgm. of amytal per kgm. intraperitoneally or nembutal as above) were allowed to respire air or oxygen from a spirometer of six liter capacity through a closed system. A current of oxygen, adjusted by means of a flow-meter, entered the spirometer through a special inlet tube in order to replace the oxygen consumed by the dog. Measured amounts of pure carbon monoxide were fed periodically into the inspiration line of the system. Blood samples were withdrawn after each administration of the gas and the concentration of carbonyl hemoglobin, HbCO, determined spectrophotometrically by the method of Drabkin (1944).

The dogs were kept at levels between 75 and 85 per cent of HbCO in the blood for periods of time ranging from 50 to 70 minutes. Of six dogs, subjected to this procedure, two developed respiratory failure after 50 to 60 minutes of exposure. However, breathing returned quickly upon the application of manual artificial respiration. In a third animal (expt. 9 of table 5), probably because of too rapid

administration of carbon monoxide, respiration stopped 10 minutes after the HbCO concentration had reached 77 per cent and cardiac failure followed three minutes thereafter. The brain biopsy was secured 6 minutes after the death of the dog. In 3 of the dogs the biopsies were performed aseptically in order to save the animal for the study of permanent alterations which might occur in the period following recovery from the severe carbon monoxide anoxia. In these instances 30 to 80 minutes elapsed between the end of exposure and the removal of the brain specimen.

Production of anoxia by temporary arrest of the blood circulation. The blood circulation of cats, under intratracheal ether anesthesia, was arrested abruptly through occlusion of the pulmonary artery by means of a clamp as described by Weinberger, Gibbon and Gibbon (1940). The clamp was released after varying intervals of time ranging from 4.5 to 9 minutes. The time between the release of the clamp and the removal of a sample of brain for analysis varied between 19 minutes and 8 days in the individual experiments.

The procedure outlined by Weinberger et al. was followed, with the exception that the preliminary thoracic operation was omitted in the animals which were to be sacrificed within one hour after the restoration of blood flow. During this period artificial respiration was applied by means of intermittent insufflation of air through the intratracheal catheter.

Technique of biopsy. In first and second biopsies, samples were taken from the parietal areas of the brain. These samples included portions of the suprasylvian and ectosylvian gyri. In the individual animals of the different groups, the first biopsy (control) was performed alternately upon the left and right sides. In third biopsies the site was anterior to that of the first biopsy. The sample was from the sensori-motor area on the supero-lateral aspect of the brain. In all biopsies the same type of anesthesia was used as in the corresponding procedure for the production of anoxia. When the animal was to be saved for further studies the head was prepared aseptically. A large scalp flap was turned down to expose the temporal muscle. The latter was divided at its insertion just lateral to the mid-line and the calvarium exposed. The bone was trephined and the opening enlarged with a rongeur to expose the parietal area. The dural flap was then opened and a piece of brain excised by means of a small scalpel. When survival of the animal was desired bleeding from the brain vessels was controlled by means of electro-coagulation and light pressure with moist cottonoid sponges. In the first operations the dura was repaired, but this procedure proved difficult and tedious. In later operations the dural flap was replaced but not sewn, with good operative results. In the majority of animals sulfanilamide powder was liberally sprinkled through all layers while the wound was closed. This seemed to be an important point in preventing infections. The animals withstood the operative procedure extremely well. In 39 biopsies only 3 animals were lost. The surviving animals appeared to be bright and alert on the first post-operative day.

The analysis of the brain specimen. Immediately after its surgical removal the sample of brain was dropped into a chilled moist chamber, a cap-style weighing

bottle, with paraffin coated internal walls and with saline moistened cotton placed in the cap. The chamber was stored in a closed jar immersed in cracked ice.[2]

After 10 minutes of storage a slice of about 3 mm. thickness was cut from the center of the piece across the gyri and fixed in formalin for histological examination. If the sample was sufficiently large, an additional slice was saved for the determination of the water content of the gray and the white matter.

The remainder of the piece was minced in a chilled weighing bottle by means of a stainless steel microspatula for a period of 2 minutes. One or two samples of well mixed mince were then withdrawn for determination of the water content. A weighed amount of the remaining mince was transferred to a homogenizer tube of the Potter-Elvehjem type (1936). Nine milliliters of warm (37°C.) stock Ringer's solution[3] were added per gram of mince, and the mixture homogenized for $1\frac{1}{2}$ minutes at 37°C.

Oxygen consumption was measured in air at 37.5°C. by means of the Warburg technique. For the measurement of the respiratory quotient the first method of Dickens and Šimer (1930) was used. The main compartment of the manometric vessels contained the following solutions: 1 ml. of homogenate, 0.4 ml. of 0.11 M sodium phosphate buffer (pH 7.35), 0.2 ml. of solution of substrate and 0.4 ml. of either stock Ringer's solution or stock Ringer's solution supplemented with thiamine hydrochloride. The final concentration of added constituents was M/100 for glucose, M/50 for pyruvate, and M/69,000 (10 micrograms per vessel) for thiamine hydrochloride.

Oxygen uptake and carbon dioxide output were measured over a period of 2 hours, 15 minutes being allowed for temperature equilibration in the water bath. The symbol Q_{O_2} as used here denotes microliters, μl, of oxygen consumed per gram of wet tissue per hour and refers to the average rate over the 2 hour experimental period. The rate over the first 30 minutes was approximately 20 per cent higher than in the last 30 minute period.

RESULTS. *Action of thiamine upon oxygen consumption of normal brain suspensions supplemented with pyruvate.* Banga, Ochoa and Peters (1939) found that in suspensions of ground brain from pigeons deficient in vitamin B_1 the oxidation

[2] Exploratory experiments on the influence of storage upon the respiratory activity of gray matter from excised cat hemispheres revealed that the activity was preserved for periods of at least 90 minutes in the chilled wet chamber (inside temperature approximately 4°C.). Storage at room temperatures between 20 and 25° also appeared to be well tolerated whereas at higher temperature, such as frequently encountered during the summer months, definite alteration in respiratory activity occurred.

[3] The composition of this solution was similar to that previously described for "Stock Ringer's solution" (Rosenthal, Bowie and Wagoner, 1941) except for the substitution of an equal volume of 0.9 per cent sodium chloride solution in place of isotonic calcium chloride. In the absence of calcium the initial rate of oxygen consumption of homogenized feline gray matter was 20 per cent higher than in presence of this cation, although the rate with calcium was constant for a period as long as 4 hours. The depressing effect of calcium was found to be smaller and its stabilizing effect more pronounced than in the experiments reported by Elliott and Libet (1942) with rat brain. In homogenizing the tissue at 37°C. we followed the directions of these authors.

of pyruvate was stimulated by the addition of thiamine diphosphate (cocarboxylase) while free thiamine failed to produce a response. In slices or minced brain preparations, on the other hand, free thiamine was much more effective than an equivalent amount of the phosphorylated compound. These authors suggested that broken cells, in contrast to intact cells, were incapable of converting the vitamin into the coenzyme, and that unbroken cells were practically impermeable to the phosphorylated compound.

TABLE 1

Effect of thiamine upon the respiration of brain suspensions supplemented with M/50 pyruvate

Mean values ± Standard error*

GROUP	TYPE OF ANIMAL	O_2 CONSUMPTION		RESPIRATORY QUOTIENT		
		No. of expts.	Change with thiamine	No. of expts.	Without thiamine	Change with thiamine
	Pigeon					
			per cent		*R.Q.*	*per cent*
a	Control	1	− 1.6	1	1.116	+1.5
b	B_1 deficient	4	+24.9 ±2.66	2	1.066 ±0.003	+5.8 ±0.16
	Dog					
a	Control	22	+0.3 ±0.38	16	1.156 ±0.004	−0.2 ±0.47
b	CO†; direct‡	6	−0.8 ±0.55	3	1.112 ±0.010	+3.5 ±0.72
c	CO†; recovered§	3	−1.5 ±0.66	2	1.135 ±0.014	+1.7 ±1.10
d	Shock‖	3	±0.0 ±0.09	2	1.145 ±0.000	+1.4 ±0.45
	Cat					
a	Control	20	−0.1 ±0.47	21	1.144 ±0.005	+1.0 ±0.32
b	Arrest¶; direct‡	6	+1.3 ±1.07	6	1.155 ±0.010	−0.1 ±0.65
c	Arrest¶; recovered§	2	−1.0 ±0.95	2	1.114 ±0.014	+3.0 ±2.6

* Standard error $= \pm\sqrt{\dfrac{\Sigma d^2}{n(n-1)}}$.

† CO—exposed to carbon monoxide.

‡ Direct—determination within 2 hours after exposure to anoxia.

§ Recovered—determination several days after exposure to anoxia.

‖ Shock—irreversible hemorrhagic shock.

¶ Arrest—temporary arrest of blood circulation.

From table 1 it is evident that thiamine in a concentration of 1.4×10^{-5} M increased the respiratory rate of isotonic brain suspensions (homogenates) of avitaminotic pigeons by 25 per cent. One-tenth of this concentration produced an increment of 20 per cent.[4] Concentrations of thiamine diphosphate[5] equivalent to the above produced increments of only 16 per cent and 9 per cent re-

[4] Unpublished experiments.

[5] We are indebted to Merck and Company, Inc., Rahway, New Jersey, for a gift of thiamine diphosphate.

spectively.[4] Hence the type of tissue preparation employed in this study behaved more like intact cells than broken cells. This result appears to corroborate Elliott and Libet's conclusion (1942) that isotonic brain suspensions are very suitable for metabolic studies.

A further inspection of table 1 reveals that thiamine failed to produce a significant increase of the respiratory rate of brain suspensions from the dogs and cats which had been subjected to the different types of anoxia which were investigated. A presentation of individual experiments seemed unnecessary since the range of variation (± 2 per cent) coincided with the reproducibility of duplicate determinations. To rule out the possibility that suspensions of brain from anoxic animals might behave like "broken" cell suspensions mentioned above, tests with thiamine diphosphate were done upon 3 dogs of group b (table 1), 1 dog of groups c and d, and 2 cats of group b, but no stimulation was observed.

These results demonstrate clearly that anoxia does not produce an uncomplicated type of thiamine deficiency such as we find in pigeons with nutritional avitaminosis. They do not rule out, however, the possibility that there may have occurred a loss of thiamine together with other enzymic factors which are required for the complete oxidation of pyruvate. The oxidative decarboxylation dependent on co-carboxylase yields a respiratory quotient of 2.0 ($CH_3COCOOH + \frac{1}{2}O_2 \rightarrow CH_3COOH + CO_2$). Probably, some additional decarboxylation of pyruvate takes place in anaerobic reactions which also depend upon co-carboxylase. Thus, addition of thiamine to a brain depleted of co-carboxylase as well as of enzymic factors required for the completion of pyruvate oxidation should yield a R.Q. above the normal level.

Table 1 also shows that the respiratory quotients of brain suspensions from control animals were of the order of 1.15, i.e., approximately 4 per cent below the theoretical value (1.2) for complete oxidation of pyruvate to CO_2 and H_2O. This may indicate that pyruvate did not suppress completely the oxidation of certain preformed tissue substrates that yielded a lower R.Q. It will be noticed that there was a considerable diminution of the R.Q. in the brain of B_1 avitaminotic pigeons in which the utilization of pyruvate is known to be diminished. The slight lowering of the R.Q. in the experimental group b of dogs and c of cats although perhaps indicative of a mild thiamine deficiency, is of questionable significance since in two animals of these groups similar low values were found before the exposure to anoxia. The addition of thiamine in these experiments restored the R.Q. to the control level but not higher. Thus there was no evidence in support of the view that anoxia affected any components of the pyruvate oxidase system.

The respiratory quotient with glucose as substrate. Although we found an increased and stabilized oxygen uptake in the presence of glucose (data not presented), which is evidence of the oxidative utilization of the sugar by brain suspensions from anoxic animals, information seemed desirable as to the completeness of the oxidation. The respiratory quotient with glucose as substrate was therefore studied in the experimental series. The results are summarized in table 2. For details of experimental procedure table 5 should be consulted.

It may be seen from the data in table 2 that in brain suspensions from first biopsies upon normal animals (dogs a and cats a) the respiratory quotient was slightly below unity, a result frequently obtained in studies with nervous tissue *in vitro* (Elliott, McNair Scott and Libet, 1942). The coefficient of variation in these groups was ±1.3 per cent. In the dog (groups b and c), the respiratory quotients of the material from subsequent biopsies upon individual brains checked within ±1.6 per cent with the result of the first biopsy, while in the cat (group b) differences ranging from −4.9 to +1.7 per cent were found. In the two cats with negative deviations of 4 to 5 per cent the interval between the two

TABLE 2

The respiratory quotient of brain suspensions supplemented with M/100 glucose

Mean values ± Standard errors

GROUP	NO. OF ANIMALS	FIRST BIOPSY	SUBSEQUENT BIOPSIES			
		R.Q.	Type of biopsy	Interval after 1st biopsy	Type of anoxia*	Change in R.Q.
				Dogs		
				days		*per cent*
a	10	0.966 ±0.004†				
b	3	0.952 ±0.006	2nd	18, 28, 43	none	+0.6 ±0.53
c	2	0.958 ±0.002	3rd	23, 49‡	none	−0.1 ±1.45
d	3	0.968 ±0.004	2nd	24, 34, 40	CO; direct	−2.9 ±0.04
e	2	0.968 ±0.007	3rd	41, 45§	CO; recovered	−1.2 ±1.20
f	1	0.984	2nd	19	Shock	−3.4
				Cats		
a	19	0.953 ±0.003†				
b	4	0.948 ±0.006	2nd	6, 7, 14, 16	none	−1.8 ±1.55
c	5	0.952 ±0.004	2nd	7, 9, 9, 20, 106	Arrest; direct	−4.1 ±1.69
d	2	0.957 ±0.008	2nd	18, 22	Arrest; recovered	−0.8 ±4.50

* Abbreviations have the same meaning as in table 1.

† The coefficient of variation, $(\pm\sqrt{\frac{\Sigma d^2}{(n-1)}}/\text{mean})\cdot 100$, was ±1.3 per cent.

‡ 5 and 6 days after 2nd biopsy.

§ 5 and 7 days after 2nd biopsy.

‖ 4 and 8 days after temporary arrest of the circulation.

biopsies was 6 to 7 days in comparison with 14 to 16 days in the other two animals which yielded values that agreed as closely as those of the normal dogs.

Exposure of dogs to carbon monoxide (d) or to hemorrhagic shock (f) resulted in a very slight decrease of the respiratory quotient. The lowest R.Q. value obtained in group d was 0.93. It seems noteworthy that the decrease following irreversible hemorrhagic shock was not more pronounced than the decrease after exposure to carbon monoxide where quick recovery was the rule. This suggests that hypoxia interferes little, if at all, with glucose utilization in the brain.

Arrest of cerebral circulation did not produce uniform responses. In 2 cats

of group c, table 2 (cf. table 5, nos. 20 and 22) no change of the R.Q. of brain was found although in one of them restoration of normal circulation did not take place until 15 minutes after release of the pulmonary occlusion, while in the other deep coma of decerebrate type had been present for over 2 hours at the time of biopsy. The brain specimens of the remaining three cats of group c yielded respiratory quotients of 0.914, 0.890, and 0.864 respectively, corresponding to reductions of 5.3, 6.5, and 8.5 per cent from the control values. In two of these animals (cf. table 5, nos. 17 and 18) restoration of blood flow was imperfect and the brain specimens were obtained about 10 minutes after complete cardiac failure. However, in the third animal (cf. table 5, no. 21) in which the greatest depression was observed, circulation returned promptly after release of the pulmonary occlusion.

A brain sample from one[6] of the two cats of group d of table 2, (cf. table 5, nos. 23 and 24), obtained 4 days after recovery from circulatory arrest, showed a lowering of the respiratory quotient by 5 per cent (R.Q. = 0.914) while a specimen from the other animal analyzed 8 days after recovery failed to reveal a significant change of the R.Q. These results suggest that stagnant anoxia may lead to a depression of glucose utilization. This effect seems to be related to the severity of the anoxia as well as to other undefined factors in the animal.

Rate of oxygen consumption. The experimental results which have been presented demonstrated that, in general, the quality of the respiratory metabolism of cerebral tissue was not materially altered by exposure of the animals to severe anoxia. It remained, however, to be seen whether the respiratory metabolism had been altered quantitatively.

The accuracy of estimating the respiratory activity of the brain suspensions was limited by the circumstance that the samples obtained by our method of biopsy were not constant in the proportion of gray and white matter. These two constituents of cerebral tissue differ greatly with respect to metabolic activity (Holmes, 1930; Krebs and Rosenhagen, 1931) and water content (Pilcher, 1937).

In three anesthetized cats both cerebral hemispheres were excised and the gray matter and white matter separated. The rate of oxygen consumption was then determined as has been described. With both glucose and pyruvate the ratio, $r = Q_{O_2}^{Gr}/Q_{O_2}^{Wh}$, of respiration of gray matter to respiration of white matter was found to lie between 3.5 and 4.8. The mean value was 4.

The gray matter content, Gr, of a biopsy specimen, S, the wet weight of which is taken as 1.0 may be computed from the equation (1).

$$Gr = [1 - (R^{Wh}/R^{S})]/[1 - (R^{Wh}/R^{Gr})], \tag{1}$$

where R^{S} is the ratio, wet weight/dry weight, in the specimen as a whole and R^{Gr} and R^{Wh} are the corresponding ratios in gray matter and white matter

[6] This animal showed loss of responsiveness, had lost interest in its environment, had anorexia, and required forced feeding. The motor functions remained intact. With reference to histological alterations compare with the "Comment." In the second cat of this group neither alterations of behavior nor histological changes were found. The only neurological defect was absence of the placing reactions.

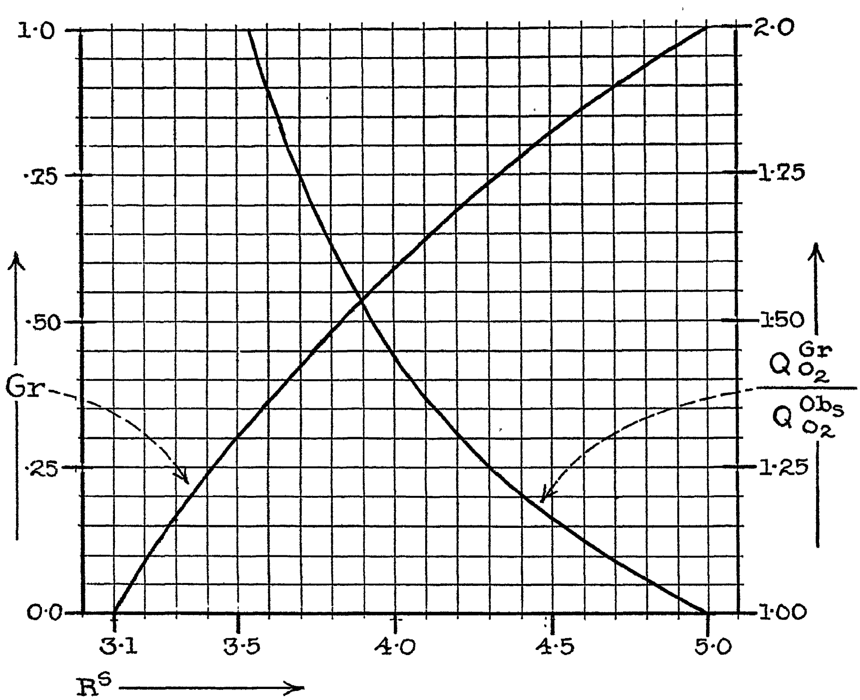

Fig. 1. Curves for computing gray matter content (Gr) and respiration of gray matter ($Q_{O_2}^{Gr}$) from the observed respiration ($Q_{O_2}^{Obs}$) and the ratio of $\frac{\text{wet}}{\text{dry}}$ weight (R^S) of brain samples.

$$Gr = \frac{1 - \frac{3.1}{R^S}}{1 - \frac{3.1}{5.0}} = \frac{1 - \frac{3.1}{R^S}}{0.380}$$

$$\frac{Q_{O_2}^{Gr}}{Q_{O_2}^{Obs}} = \frac{1}{Gr + \frac{1 - Gr}{4}}$$

TABLE 3

The ratio of wet weight to dry weight in gray matter (R^{Gr}) and in white matter (R^{Wh})

SPECIES	NO. OF ANIMALS	NO. OF DETERMINATIONS	R^{Gr} OR R^{Wh}		
			Mean	C.V.*	S.E.†
Gray matter (R^{Gr})					
				per cent	
Dog	11	23	5.04	±3.64	±0.038
Cat	10	16	4.98	±4.05	±0.051
Dog and cat	21	39	5.01	±3.84	±0.031
White matter (R^{Wh})					
Dog	11	21	3.12	±5.15	±0.043
Cat	9	14	3.08	±5.70	±0.046
Dog and cat	20	35	3.10	±5.00	±0.026

* C.V. = coefficient of variation.
† S.E. = standard error.

respectively. While R^S could be determined for all brain specimens used in the measurement of metabolism, R^{Gr} and R^{Wh} could not. Therefore, in computing Gr we have used the mean values of R^{Gr} and R^{Wh} which are listed in table 3. The curve Gr of figure 1 serves to illustrate the relationship between Gr and R^S obtained by this method.

The rate of oxygen consumption per gram of wet weight of gray matter, $Q_{O_2}^{Gr}$, is obtained from the observed rate of oxygen consumption, $Q_{O_2}^{Obs}$, using equation (2).

$$Q_{O_2}^{Gr} = Q_{O_2}^{Obs}/[Gr + (1 - Gr)/r]. \tag{2}$$

The other curve of figure 1 shows the relationship between the ratio $Q_{O_2}^{Gr}/Q_{O_2}^{Obs}$ and the water content of the brain samples, expressed in R^S values. In the range met with in practice, i.e., for R^S values greater than 4 or Gr values greater than 0.6, variations of r, even as large as 25 per cent, would have only negligible effect on the value of $Q_{O_2}^{Gr}$. The accuracy of $Q_{O_2}^{Gr}$ depends whether the mean

TABLE 4

Observed rates of respiration and rates of respiration computed for gray matter with M/100 glucose ($Q_{O_2}^{G}$) or M/50 pyruvate ($Q_{O_2}^{Pyr}$)*

Normal animals

Mean values ± coefficients of variation

ANIMAL		NUMBER OF EXPTS.	$Q_{O_2}^{G}$		$Q_{O_2}^{Pyr}$†	
Species	No.		Observed	Computed	Observed	Computed
Dog....	11	16	871 (±10.8%)	1049 (±8.3%)	925 (±8.6%)	1116 (±7.8%)
Cat	16	21	835 (±8.6%)	1053 (±7.2%)	927 (±7.6%)	1159 (±5.4%)

* $\mu l O_2$/g. wet weight/hour. Mean rate over 2 hour period.

† The individual values are means of the rates obtained with and without thiamine which agreed within the limit of technical error.

values of R^{Gr} and R^{Wh} used in computing Gr are truly representative, as well as on the accuracy of determining R^S in the individual specimen.

Results of determinations of the respiratory activity in brains from normal animals are summarized in table 4. It will be seen that computation of the rates per gram of gray matter instead of per gram of original sample results in a slight reduction of the coefficient of variation in the experimental series. This reduction is accounted for by the elimination of the extreme deviations by our method. However, the scatter range of the majority of values remained unchanged. At present it cannot be decided whether the extent of this scatter is a consequence of unavoidable imperfections in technique or of real differences in the respiratory activity of the individual brain specimens. That our method of computation is basically sound is substantiated by the finding that direct determinations of $Q_{O_2}^{G}$ and $Q_{O_2}^{Pyr}$ in the gray matter from 5 cats yielded mean values of 1092 and 1198 respectively, values in excellent agreement with the computed means recorded in table 4.

In table 5 the two methods of calculation have been applied to data on the respiratory activity of different brain specimens from individual dogs and cats.

TABLE 5

Respiratory activity of different brain samples from individual animals

EXPT. NO.	ANIMAL NO.	SITE AND ORDER OF BIOPSIES	INTERVAL BETWEEN BIOPSIES	EXPOSURE TO ANOXIA			TIME FROM END OF EXPOSURE TO BIOPSY	$\Delta Q_{O_2} \times 100$*			
				Type	Details‡	Duration		With glucose		With pyruvate	
								Observed	Gray matter	Observed	Gray matter
Dogs											
			days			*mins.*		%	%	%	%
1	15	R, L	43	None				−6.0	+11.0	−7.5	+9.5
2	15	R, Rant	49	None				−17.5	−0.5	−12.5	+5.5
3	18	L, R	18	None				−14.0	−4.0	−2.5	+8.5
4	18	L, Lant	23	None				−10.0	−2.5	−5.5	+2.5
5	8	L, R	34	CO	HbCO 74–79%	50	40 mins.	+18.5	−3.5	+14.5	−6.0
6	8	L, Lant	41	CO			7 days	+5.5	−4.5	+2.5	−7.5
7	13	R, L	40	CO	HbCO 77–93%	60	80 mins.	+6.0	−3.0	+8.5	−2.5
8	13	R, Rant	45	CO			5 days	+10.0	4.0	+4.5	−1.0
9	12	R, L	24	CO	HbCO 77% up	10	9 mins.§	−25.0	−24.5	−21.0	−20.5
10	10	R, L	14	Shock	BP 30 mm.	135	120 mins.‖			−2.5	+23.5
11	11	L, R	19	Shock	BP 32 mm.	180	0 mins.	−7.0	+5.5	−6.5	+7.0
Cats											
12	3	R, L	5	None				−13.0	+1.5	−9.0	+4.5
13	14	R, L	6	None				−7.5	−5.0	−10.0	−7.5
14	16	L, R	7	None				−1.5	−0.5	−9.0	−8.0
15	20	L, R	14	None				−8.5	+6.5	−13.0	+1.0
16	21	R, L	16	None				−5.5	−1.0	−5.5	−1.5
17	7	R, L	7	Arrest	Occl. 5.3 mins.¶	7 to 40¶	8 mins.	−26.5	−26.5	−19.0	−19.5
18	24	L, R	109	Arrest	Occl. 9.0 mins.¶	9 to 35¶	11 mins.	−21.0	−24.0	−8.5	−12.0
19	23	R, L	23	Arrest	Occl. 8.0 mins.	9	16 mins.	−2.5	+9.0	−5.5	+5.5
20	4	L, R	9	Arrest	Occl. 6.0 mins.	20	23 mins.	−6.5	−9.5	−4.5	−7.0
21	1	L, R	9	Arrest	Occl. 6.2 mins.	5.2	27 mins.	−13.5	−21.0	−17.5	−24.5
22	18	L, R	20	Arrest	Occl. 5.0 mins.	5.7	136 mins.	−15.5	−25.5	−13.0	−23.5
23	6	R, L	18	Arrest	Occl. 4.5 mins.	3.4	4 days	−10.5	−11.5	−8.5	−9.5
24	19	L, R	22	Arrest	Occl. 6.2 mins.	6.5	8 days	−10.0	−10.5	−4.5	−5.5

$$*\Delta Q_{O_2} \times 100 = \frac{(Q_{O_2} \text{ subsequent biopsy}) - (Q_{O_2} \text{ first biopsy})}{Q_{O_2} \text{ first biopsy}} \times 100.$$

† "R" and "L" denote parietal areas of right and left hemisphere respectively, the first letter always designating the site of the first (control) biopsy. The subscript "ant" indicates an area anterior to the site of the first biopsy.

‡ "HbCO" and "BP" indicate respectively concentration of carbon monoxide hemoglobin as percentage of the total hemoglobin and blood pressure in mm. of mercury for the period of time given in the next column to the right. "Occl." designates time of occlusion of the pulmonary artery. The corresponding figures sub "Duration" give the period of interruption of retinal circulation.

§ 6 mins. after cardiac failure.

‖ 120 mins. after reinfusion of the blood. The blood pressure had drifted back to 40 mm. Hg.

¶ Although cardiac action remained weak and irregular after release of the occlusion some circulation was sustained by means of heart massage and infusion of saline with epinephrine. This was evidenced by regressing cyanosis of the mucous membranes. The specimens were obtained 8 and 11 minutes after complete cardiac failure.

The percentage difference in the oxygen consumption, $\Delta Q_{O_2} \times 100$, between the control and subsequent biopsies is recorded.

From the data on normal animals (expts. 1 to 4 and 12 to 16) it is evident that, when the respiratory activity is calculated upon the basis of gray matter, $\Delta Q_{O_2} \times 100$ is diminished in most instances, namely, in 15 out of 18 determinations. This may be seen from a comparison of the values for gray matter with the corresponding observed values. Values of $\Delta Q_{O_2} \times 100$ greater than 10 per cent appear to be beyond the limit of experimental error and may be utilized as evidence of abnormality. Negative values greater than 10 per cent, indicative of depressed respiratory activity of the brain, were encountered in experiments with five animals subjected to complete anoxia. Such experiments included 4 instances of circulatory arrest (expts. 17, 18, 20, 21) and one instance (expt. 9) of carbon monoxide anoxia, in which the specimen was obtained 9 minutes after respiratory failure of the dog. No depression was found under conditions of hypoxia, such as uncomplicated carbon monoxide poisoning and hemorrhagic shock.

It is probable that the single positive value of $\Delta Q_{O_2} \times 100$ in gray matter greater than 10 per cent (expt. 10) which could be taken as indicative of stimulation of the respiratory activity by irreversible hemorrhagic shock is in error owing to the dehydration of the brain in situ. At biopsy of this animal's brain the distance between the dura and brain surface appeared to be extraordinarily wide. Correspondingly, the water content of this brain sample ($R^s = 3.91$) was unusually low. Since our computation is based upon the standard curves, presented in figure 1, dehydration of the brain must result in an underestimation of the gray matter content of the sample and, consequently, in an overestimation of the respiratory activity. It is obvious that brain edema will produce errors in the opposite direction. This is probably the situation in experiments 21 and 22 in which the large negative values of ΔQ_{O_2} appear to be at variance with the brief period of circulatory arrest. In these instances the water content of the brain samples was considerably higher than that of their respective controls. In experiments 19 and 20, on the other hand, in which there was no increase in the water content of the samples over that of the respective controls, there was no significant change in the respiratory activity even though the periods of interrupted cerebral blood flow were longer than in experiments 21 and 22 (vide the figures sub "Duration" in table 5).

Comment. Our experiments indicate that neither irreversible hemorrhagic shock nor severe carbon monoxide hypoxia diminishes the respiratory capability of brain tissue.[7] Even after interruption of cerebral blood flow for periods of time sufficient to produce prolonged functional and, often, permanent histological alterations in the nervous system, a reduction in the respiratory activity of the brain is not a primary consequence.

Although this conclusion refers to the respiratory activity of the cerebrum only under standardized *in vitro* conditions, nevertheless it does not support the

[7] In a personal communication to one of us (D. L. D.) C. N. H. Long has recently informed us that he and his colleagues A. E. and H. Wilhelmi found that the metabolism of brain from rats in hemorrhagic shock, in contrast to the metabolism of liver, shows no alterations from normal. This is in agreement with our findings in dogs.

hypothesis that irreversibly depressed oxygen consumption of brain *in situ*, as has been reported to follow prolonged periods of cerebral anemia (Schmidt et al., 1945), is due to destruction of respiratory enzymes or coenzymes. The cause of the decreased oxygen consumption *in vivo* may perhaps be accounted for by an impaired supply of oxygen and substrates to the brain cells owing to permanent vascular changes, and/or a disturbed biocatalytic activity as a consequence of alterations in intracellular water and electrolyte content.

Histological examination of the brain specimens from the anoxic animals failed to reveal significant changes with the exception of a specimen removed 4 days after temporary arrest of the circulation (vide table 5, expt. 23 and footnote 6). In this case over 50 per cent of the ganglion cells were shrunken and pyknotic with the cytoplasm staining so darkly as to make the nucleus indistinguishable save for the nucleolus. Apical processes were prominent, elongated and wavy. The third layer of the cortex seemed most severely affected. Indeed here a small area of degeneration could clearly be seen, with falling out of cells leaving small vacuoles and some debris. Of the brain specimens removed several days after exposure of the animal to anoxia this was the only one in which a depressed respiratory quotient with glucose as substrate was found. The other specimens, which showed various metabolic alterations, were obtained within 2 hours following exposure of the animal to anoxia, i.e., presumably too early for histological changes to have occurred.

Drabkin et al. (1943) had found 75 per cent carbonyl hemoglobin in the blood to be a critical level with unanesthetized dogs. Pathological lesions of the brain were always seen in dogs kept for brief periods under these conditions. It therefore appeared remarkable to us that in our present experiments the dogs were able to survive without apparent ill effect appreciably longer periods (up to 85 min.) of acute carbon monoxide hypoxia at levels as high as 85 per cent (and in one instance 93 per cent) of HbCO in the blood. It may be calculated from the effect of HbCO upon the shift in the dissociation of the remaining oxyhemoglobin that at a level of 85 per cent HbCO, not 15 per cent, but only approximately 4 per cent of the hemoglobin may be considered functional from the standpoint of unloading of oxygen to the tissue. It appears highly probable to us that survival and complete recovery under our present conditions are largely due to the fact that our dogs were anesthetized and therefore at a low level of metabolism. Other interesting implications of this severe form of carbon monoxide hypoxia will be further discussed elsewhere.

SUMMARY

Oxygen consumption rates and respiratory quotients, with pyruvate or glucose as substrate in an isotonic medium were studied in brain suspensions *a*, from dogs in irreversible hypotensive hemorrhagic shock; *b*, from dogs exposed to high concentrations of carbon monoxide (75 to 85 per cent HbCO for 50 to 70 min.), and *c*, from cats subjected to temporary arrest of the circulation. In each animal biopsy specimens from the cerebral hemispheres were examined one to several weeks before exposure to the anoxic state, immediately thereafter, and/or,

occasionally, following recovery from the reversible types of hypoxia (*b* and *c* above).

Since individual biopsy specimens varied in the proportions of gray and white matter, a method has been presented for computing the respiratory activity of gray matter from the water contents of mixed samples and the mean values for water content and respiratory activity in samples of gray and white matter respectively.

In the experiments with pyruvate measurements were made both in the presence and absence of added thiamine-HCl to test whether anoxia produced a critical deficiency of this factor. Control experiments on brain suspensions from pigeons with frank neurological manifestations of vitamin B_1 deficiency showed striking increases in O_2 consumption after addition of thiamine hydrochloride.

An altered respiratory activity was found in some but not all of the brain specimens from the animals exposed to stagnant anoxia (circulatory arrest). The alterations consisted in a lowered rate of O_2 consumption with pyruvate as substrate and in diminished respiratory rates and quotients with glucose as substrate. In carbon monoxide hypoxia and in irreversible hemorrhagic shock the respiration of the brain remained essentially normal. The addition of thiamine hydrochloride was without effect in all three types of induced anoxia.

The experimental findings suggest that a destruction of respiratory enzymes or a critical deficiency of co-enzymes in the brain is not a primary consequence of anoxia nor do such factors account for the development of irreversible functional and histological alterations, that may follow anoxia.

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CRYSTALLINE VITAMIN B_c IN RELATION TO THE CELLULAR ELEMENTS OF CHICK BLOOD

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In 1943 Pfiffner and associates (1) reported on the isolation of crystalline vitamin B_c. The present paper gives a summary of our findings with respect to the rôle of this pure vitamin in its relation to the cellular blood elements. The absence of vitamin B_c in the diet of the chick produces subnormal growth, poor feathering and profound alterations in the peripheral blood. Hogan and Parrott (2), who were the first to describe the poor growth and abnormal erythropoiesis, characterized the anemia as hyperchromic and macrocytic in nature. This induction of anemia in chicks raised on a simplified ration lacking an unrecognized factor was confirmed by Miller, Briggs, Elvehjem and Hart (3). Later, Campbell, Brown and Emmett (4, 5) demonstrated that, in addition to the macrocytic, hypochromic anemia, both severe leukopenia and mild thrombopenia occurred in chicks raised on a purified diet lacking the anti-anemia principle but containing all of the other recognized B vitamins in crystalline form. Briggs, Luckey, Elvehjem and Hart (6) confirmed the presence of abnormal leukopoiesis.

Experimental. The chicks used throughout were produced from the same strain of white leghorns. They were kept in close confinement and raised on dry feed. Upon arrival in the laboratory, the day-old chicks were banded, weighed and segregated in uniform test groups. They were housed in metal brooders equipped with raised wire bottoms. All experiments were conducted in an air-conditioned room maintained at 84 to 86°F. Tap water and the ration were given ad libitum. Each week observations were made as to body weight, perosis and general appearance of the birds. Mortality records were also kept daily starting after the eighth day on the ration on the assumption that any deaths prior to this were not due to the effects of the experimental conditions. As the experiment progressed, some of the chicks were selected at random for hematopoietic examination. The blood was drawn from the brachial vein at the tip of the wing, using a small hypodermic needle for the incision.

The following methods were chosen for examining and classifying the chick blood cell elements: (a) for the erythrocyte and leukocyte count, the method of Wiseman as described by C. Olson (7); (b) hematocrit values were determined according to Van Allen (8); (c) for hemoglobin, the method of Evelyn (9); (d) thrombocytes by the method of Fonió as described by R. B. H. Gradwohl (10) and (e) Wright's stain was employed for the staining of slides for the differential counts. In addition, we used as a guide in the morphological study, Burnett's

(11) colored photographs of normal chicken blood cells and the colored drawings of Oberling and Guérin (12) of normal and pathological chick blood cells.

The plan, as carried out, was to keep the chicks on three nutritive planes: (a) normal diet, (b) vitamin B_c deficient purified basal diet, and (c) the basal diet supplemented with varying amounts of the crystalline vitamin.

A. *Normal control group.* In order to study the blood picture of a normal growing chick, a commercial broiler ration (Larrowe Milling Co.) was fed to three groups of 10 chicks each. It contained 18 per cent protein, 2.5 per cent fat, 7.0 per cent crude fiber, and 47.5 per cent nitrogen-free extract. While the protein content was perhaps low, no attempt was made to reinforce the ration. The animals were started on this ration on three successive weeks. The results are summarized in table 1.

TABLE 1

A. Normal chick blood

AGE OF CHICKS, DAYS	1	3	7	9	14	21	28
Number of chicks	27	10	26	15	23	22	20
Body weight, grams	38		54	63	85	152	207
Hematocrit, vol. %	33.2	30.3	32.8	31.5	32.3	34.9	31.2
Hemoglobin, gram %	8.02		8.56	7.36	7.95	8.30	7.74
Erythrocytes, mils./cmm.	2.28	2.31	2.37	2.30	2.32	2.42	2.27
Leukocytes per cmm.	19,580	23,740	26,577	28,648	28,640	29,950	29,935
Lymphocytes	12,413	16,381	19,647	21,170	20,380	21,780	22,406
Monocytes	470	546	797	745	1,071	1,021	688
Eosinophils	705	617	520	533	573	614	449
Basophils	529	475	736	1,063	902	1,144	659
Heterophils	5,463	5,721	4,877	5,130	5,714	5,391	5,733
Thrombocytes per cmm.	29,630	24,940	29,400	32,137	26,090	31,754	31,180

B. *Negative control group.* Three groups of 15 chicks each were started concurrently with the normal group and given the following basal ration during the next 4 weeks on test. The purified diet had the following composition per 100 grams: purified casein, 25 grams; gelatin, 10 grams; l-cystine, 0.3 gram; cornstarch, 52.4 grams; cellulose, 3 grams; lard, 4 grams; salts (13), 5 grams; manganese sulfate (4 moles of water), 0.1 gram; choline chloride, 0.2 gram; thiamine, 0.4 mgm.; riboflavin, 0.8 mgm.; pyridoxine, 0.6 mgm.; sodium pantothenate, 1.1 mgm.; nicotinic acid, 2.0 mgm.; i-inositol, 50 mgm.; p-aminobenzoic acid, 15 mgm.; vitamin A, 1600 U.S.P. units; vitamin D (natural), 160 U.S.P. units; mixed tocopherols (25 per cent alpha), 24 mgm.; and vitamin K, 5 mcg. Crystalline biotin in 0.005 N NaOH solution was given by pipette twice a week at a level equivalent to 1.5 mcg. per chick per day.

Description of the vitamin B_c deficiency syndrome in chicks. Birds fed the basal deficient ration became progressively lethargic after 7 to 10 days. Their wings

began to droop by the end of the first week. In general, immature development of the feathers was a noticeable feature, progressing slowly after the second week until it became very pronounced after five weeks. For example, the lamellae and the rachis were particularly involved. The enamel on the shaft, especially in the median area, gradually disappeared leaving a weakened feather which easily snapped off when a slight pressure was applied to it. This phase of the syndrome did not appear to be as severe in our chicks as reported by Briggs et al. (6). Other than a general dryness of the skin in the four weeks or older birds, no dermatologic effects were observed. The incidence of perosis among the chicks fed the basal diet was extremely low, being about 1 per cent. The chicks grew normally for the first eight to ten days; thereafter, the growth rate dropped appreciably. The mortality of the anemic chicks, receiving little if any of the vitamin B_c, was extremely high, averaging 50 per cent in four weeks.

TABLE 2

B. Vitamin B_c deficient chick blood

AGE OF CHICKS, DAYS	3	7	9	14	21	28
Number of chicks	10	15	18	22	16	25
Ave. body weight, grams		57	63	66	84	99
Hematocrit, vol. %	30.2	29.9	26.1	23.5	22.6	15.0
Hemoglobin, gram %		7.64	6.34	6.27	5.85	4.76
Erythrocytes, mils./cmm.	2.17	2.09	1.96	1.70	1.33	0.93
Leukocytes per cmm.	19,780	25,780	18,520	13,686	9,047	7,690
Lymphocytes	11,215	19,386	12,840	8,997	3,736	1,756
Monocytes	336	825	432	230	85	68
Eosinophils	574	619	463	335	611	274
Basophils	1,246	704	629	516	260	197
Heterophils	6,409	4,246	4,156	3,608	4,355	5,395
Thrombocytes per cmm.	26,350	29,570	24,913	24,541	18,487	18,020

After certain intervals of time on the ration a number of birds were selected at random for blood sampling. The data are given in table 2.

C. *Vitamin B_c supplemented group.* Crystalline vitamin B_c was added to the basal ration in varying amounts and fed to day-old chicks. Seven groups of 4 to 7 chicks each were given the test diets for 27 days and the observations on blood response were made at this time. This series was run in duplicate on 2 successive hatches. The first test has been reported previously (4) whereas a complete summary of the 2 assays is shown in table 3.

A. *Observations on the blood of the normal control chicks.* The blood constituents of the normal chick (table 1) remained on a fairly constant level during the first 28 days of the test. Only an increase in the number of leukocytes, accounted for in the most part by lymphocytes, gave evidence of the dynamic state of the blood.

Morphologically, full maturity of the cells occurred after 2 to 3 weeks. Up to that time slight to moderate polychromasia and basophilia were demon-

strated in the nucleated erythrocytes along with a small number of immature forms Occasionally poikilocytosis and anisocytosis were observed.

In the white cells excessive granulation and slight swelling of the leukocytes were observed in some of the day-old chicks. A few eosinophilic myelocytes appeared after a week or so. On the whole, however, the white blood cells and the thrombocytes were essentially normal for the 28 days under examination.

Statistically the coefficients of variation for normal red, white, differential and thrombocyte counts agreed fairly well with those reported by Olson (7). See table 4.

B. *Observations on the blood of the negative control.* The blood picture of chicks fed the basal vitamin B_c deficient diet (table 2) was similar for the first 7 days to that produced in chicks given normal (broiler) ration. Thereafter, disorders in the blood developed which became progressively more severe. Thus, the red

TABLE 3

C. The cytopoietic influence of crystalline vitamin B_c after twenty-seven days

LEVEL/100 GRAMS OF RATION	0	5 mcg.	10 mcg.	20 mcg.	40 mcg.	100 mcg.	200 mcg.	400 mcg.
Number of chicks	5	9	4	5	7	12	8	10
Ave. body weight, grams	98	97	109	120	132	161	181	202
Hematocrit, vol. %	15.2	19.7	20.5	*31.0**	30.6	30.2	33.1	30.7
Hemoglobin, gram %	3.38	4.97	5.12	6.78	7.25	*7.67**	8.65	8.05
Erythrocytes, mil./cmm.	0.89	1.30	1.17	1.92	1.99	2.08	*2.27**	2.12
Leukocytes per cmm.	9,400	8,860	11,630	18,420	16,300	26,950	*30,540**	24,440
Lymphocytes	1,109	3,258	4,038	6,668	4,523	17,337	19,470	16,961
Monocytes	0	56	87	442	272	1,145	1,261	855
Eosinophils	207	224	407	332	217	270	6	244
Basophils	132	203	378	774	163	539	774	415
Heterophils	7,952	5,119	6,720	10,204	11,125	7,659	9,029	5,965
Thrombocytes per cmm.	18,700	21,530	22,020	*32,450**	36,580	38,100	35,900	32,620
Mortality, %	58	36	67	55	22	25	20	0
Incidence of perosis, %	0	0	0	0	0	8	25	20

* These values are comparable to those obtained with chicks raised on a commercial broiler ration (table 1).

blood cell volume after 4 weeks decreased in some instances to 5–6 per cent; the erythrocyte count dropped to 310,000 cells per cmm. in one chick; and the leukocyte counts of 3,000 to 4,000 per cmm. were not uncommon. One of the remarkable features of this dyscrasia was the maintenance of the absolute number of heterophiles. During the 28 days of observation, there was a relative increase in heterophiles from 28 per cent to 70 per cent, but, as the data indicate, there was no significant increase or loss in actual number.

The morphology of the cells was found to be decidedly changed from the normal starting on or about the ninth day. Immature red blood cells increased in number with more polychromatophilia and basophilia present. Macrocytosis with a greater variety of sizes and shapes appeared on about the 14th day. By the 21st day, numerous normoblasts, pronormoblasts and myeloblasts were in evidence; active mitotic figures became more common. Ultimately at 28 days the nucleated red blood cells presented a moderate to marked anisocytotic and

poikilocytotic picture. Also polychromasia and basophilia, along with macrocytosis, were in evidence as a general rule rather than the exception. In addition, the nuclei of many of the immature cells were eccentrically placed while the breaking of nuclear chromatin into fragments within the cell was frequently seen.

Of the twenty-five animals surviving on the basal deficient ration, table 2, four did not develop as severe an anemia in the four weeks as the others. Their hemoglobin values ranged from 5.88 to 7.53 grams per cent, averaging 6.84 grams per cent. The red count ranged from 1.32 to 1.59 million cells per cmm. with a mean of 1.47 while the hematocrits varied from 23 to 28 vol. per cent, averaging 25.5 vol. per cent. However, the morphology of their cellular elements was as characteristically marked as the rest of the group.

The leukocytes deviated considerably from the normal pattern. The excessive granulation and swelling of the cells observed in the blood picture of the day-old chick disappeared after a day or two in a normal manner. About the ninth day and thereafter, however, the distribution, size, number, and staining reactions of all white cells became progressively variable as leukopenia developed. Heterophiles appeared to be aging since pyknosis and hypersegmentation were often observed. The nuclei of these cells also acquired a lighter staining characteristic, starting generally about the twenty-first day. Vacuoles developed in the cytoplasm of the lymphocytes after the fourteenth day. Moderate to severe leukopenia was a distinguishing feature in every bird in this series.

The thrombocytes underwent considerable alteration after the second week, although their actual numbers did not decrease much below a subnormal plane. Karyorrhexis and pyknosis were common. Vacuolation on the cytoplasm increased during the third and fourth weeks along with a general swelling of the granuoles. Variation in the size and shape of the cells proper was a dominant characteristic. Often it was difficult to distinguish between platelet and lymphocyte because of the increase in their size. Three of the chicks presented an essentially normal thrombocyte picture while in five other cases only very slight granulation and swelling of the cells were observed. Of the eight chicks referred to with little or no thrombopenia developing, three were previously described as being only slightly anemic.

The instability of the blood elements in the vitamin B_c deficient chicks is clearly reflected by the coefficients of variation associated with each observation, the variance being one to six times the normal values. See table 4.

C. *The influence of crystalline vitamin B_c on the cellular elements of chick blood.* The addition of 5 to 10 mcg. of pure vitamin B_c per 100 grams of basal ration (table 3) failed to prevent the appearance of a severe macrocytic, normochromic anemia in the 27 day test. The characteristic morphology of the blood pattern shown in the negative control animals was duplicated here. In the 5 mcg. group one chick of the nine had a normal blood picture, although its body weight was low, 84 grams.

Marked improvement in the thrombocyte pattern of the chicks given 20 and 40 mcg. respectively, of the crystalline B_c vitamin per 100 grams of ration, was

accompanied by a partial prevention of anemia and leukopenia, suggesting that these levels were near the critical zone. The variations in red cell size and shape were considerably reduced. Although the leukocytes were slightly abnormal morphologically, severe lymphocytopenia was still evident.

The distribution, size, shape, and staining characteristics of the blood cells in the chicks receiving 100 mcg. or more of crystalline vitamin B_c per 100 grams of basal ration were essentially normal. The approximate normal values have been italicized in table 3. This emphasizes the small amount of vitamin B_c (20 mcg.) necessary to maintain the normal red cell volume and thrombocyte count.

TABLE 4

Range and variation of results

OBSERVATION	NORMAL CHICKS		ANEMIC CHICKS		VITAMIN B_c TREATED CHICKS					
					5 mcg./100 gm.		100 mcg./100 gm.		400 mcg/100 gm.	
	Range	Coef.	Range	Coef.	Range	Coef.	Range	Coef.	Range	Coef.
Number of chicks	20		25		9		12		10	
Body weight, gram	132–255	16.2	59–150	23.9	62–155	27.5	81–215	22.7	101–255	24.5
Hematocrit, vol. %	27–36	9.7	5–28	46.0	9–28	29.4	25–35	10.6	23–35	11.4
Hemoglobin, gram %	6.42–10.07	11.8	2.35–7.53	36.9	1.94–7.65	36.4	6.50–8.81	10.3	6.74–9.10	8.2
Erythrocytes, mil. /cmm.	2.00–2.58	7.5	0.31–1.59	40.1	0.59–2.17	36.1	1.60–2.41	10.1	1.28–2.56	19.9
Leukocytes per cmm.	23,100–40,000	17.0	3,000–14,700	43.6	3,200–18,000	53.0	20,000–42,200	29.1	18,000–33,000	16.9
Lymphocytes	13,630–32,800	10.1	420–3,530	68.4	615–9,000	49.8	9,615–25,320	21.6	11,270–22,440	13.3
Monocytes	240–1,470	51.3	0–270	103.4	0–260	114.3	200–4,220	56.0	10–2,950	90.0
Eosinophils	0–900	76.7	0–470	80.3	0–540	29.6	0–520	60.0	0–540	62.9
Basophils	250–1,600	47.7	0–580	118.8	0–1,080	67.4	0–1,100	85.0	180–675	45.5
Heterophils	2,895–13,985	35.9	740–11,970	29.4	1,950–7,200	36.8	2,885–14,000	48.5	2,890–9,570	30.3
Thrombocytes per cmm.	21,000–36,700	14.3	10,000–35,900	34.5	14,700–32,550	32.0	32,800–42,000	8.1	18,300–38,400	18.9

Coef. = coefficient of variation = $\dfrac{\text{Standard deviation}}{\text{Arithmetical average}} \times 100.$

Statistical examination (table 4) of all the data for chicks receiving crystalline vitamin B_c in the diet shows that the range and the coefficient of variations of each observation of the low levels were similar to the variances noted in the negative controls, but, as the amount of vitamin B_c added to the ration increased, the response became less variable.

DISCUSSION.[1] The anemia produced in chicks fed a vitamin B_c-free diet may be of the hyperchromic type as pointed out by Hogan and Parrott (2). This classification is not too specific since we have observed hypochromic as well as hyperchromic anemia in our chicks on a purified ration. In addition, when small amounts of vitamin B_c (5 to 10 mcg. per 100 grams of ration) are fed to growing chicks, the anemia is distinctly normochromic in nature.

[1] The authors wish to express their appreciation to Dr. E. A. Sharp of the Clinical Investigation Department for his valuable comments.

It is also interesting to note that vitamin B_c in doses up to 400 mcg. per 100 grams of ration does not overstimulate the production of the cellular elements of chick blood after the normal plane is reached. As previously reported (4), 20 to 40 mcg. of the anti-anemia vitamin per 100 gm. of ration gave nearly optimum protection against anemia and thrombocytopenia. We believe from the examination of the present data, however, that, instead of 400 mcg. of vitamin B_c, 20 to 200 mcg. of the crystals per 100 grams of basal ration is sufficient for normal hematopoietic maintenance in the growing chick for the four weeks of life.

The appearance of perosis in chicks receiving the higher levels of crystalline vitamin B_c would seem to confirm the observation of Richardson et al. (14) that there is a factor concerned with perosis, other than the choline, biotin, and manganese.

SUMMARY

1. Data have been presented on the cellular blood elements of normal, vitamin B_c deficient, and vitamin B_c treated chicks.

2. Crystalline vitamin B_c prevents macrocytic anemia, leukopenia and thrombopenia in chicks during the first four weeks of life.

3. Chicks raised on a purified ration containing all of the known B vitamins in crystalline form along with ample amounts of manganese, still develop perosis. This suggests that another unknown perotic factor exists.

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THE OSMOTIC ACTIVITY OF GASTROINTESTINAL FLUIDS AFTER WATER INGESTION IN THE RAT

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Literature concerning the rate of water absorption is fairly extensive (1–9), but the problem has been approached largely from the point of view of blood dilution, tissue water load and diuresis. No organized study has been made of the osmotic tonicity changes in various regions of the gastrointestinal tract following the ingestion of water. Smirk (7), working with rats, has measured chloride concentrations following the ingestion of 5 per cent of the body weight of water. At the end of absorption (about 1 hr.) fluid in the stomach had a chloride concentration of 17.8 m.Eq. per liter, while that in the intestine showed 98 m.Eq. per liter. Karr and Abbott (10) have made chemical studies on fluid withdrawn by the "Abbott-Miller tube"[1] (11) from various sections of the gastrointestinal tract of the intact human subject. Total osmotic activity was calculated by adding the chloride and bicarbonate concentrations. In the fasting subject "duodenal" samples averaged 90 m.Eq. per liter and "jejunal" samples 122 m.Eq. per liter. Ten minutes after drinking 400 cc. of water, the "jejunal" samples fell to about 100 m.Eq. per liter.

Direct determination of the actual osmotic activity changes in different sections of the gastrointestinal tract following the ingestion of distilled water seems desirable, especially since water has been shown to damage the jejunal and ileal mucosa, both physiologically and histologically (12), and since water, administered to dogs by stomach tube, has been found to pass into a duodenal fistula as early as five to ten seconds after introduction into the stomach (13).

The present investigation was made in order to study the osmotic tonicity changes in the contents of the gastrointestinal tract resultant from the ingestion of distilled water.

Procedure. Male white rats (from Sprague-Dawley Company, Madison, Wisconsin) weighing from 225 to 275 grams were used as experimental animals. They were starved for 24 hours prior to the experiment, but were allowed water ad libitum until one hour before the experiment.

Water, amounting to two per cent of the body weight, except as otherwise noted, containing 2.4×10^{-4} grams Evans blue dye per cubic centimeter, and warmed to body temperature, was administered by stomach tube. After ten minutes the animals were anesthetized with ether. The abdomen was incised and sections of the gut clamped immediately with small-sized hemostats. Sample 1 was withdrawn from the stomach, sample 2 from the duodenum, and

[1] The fluid collected in this manner at any point is likely to be diluted with the contents of the gastrointestinal tract anywhere above that point.

sample 3 from the beginning of the jejunum to the lowest portion of the small intestine colored blue by the dye. In some cases an additional sample was withdrawn by stomach tube prior to the administration of ether. The samples were aspirated into syringes and placed in air-tight bottles kept in the refrigerator until the osmotic pressures were determined. Subsequent drainage of the gut sections added little if any additional volume. The volumes of the samples collected and the length of the gut from which they were withdrawn were recorded. Blood samples were taken anaerobically from the abdominal aorta just above its bifurcation. The blood was set aside for fifteen minutes to clot, then centrifuged

TABLE 1

Osmotic activity and chloride concentrations in gastric, duodenal and jejunal fluids removed ten minutes after administration of water by stomach tube

(Rats anesthetized with ether ten minutes after water ingestion)

RAT NO.	S1		S2		1		2		SERUM	
	O.A. m.Eq. NaCl/kg. H_2O	(Cl.) m.Eq./L.	O.A. m.Eq. NaCl/kg. H_2O	(Cl.) m.Eq./L.	O.A. m.Eq. NaCl/kg. H_2O	(Cl.) m.Eq./L.	O.A. m.Eq. NaCl/kg. H_2O	(Cl.) m.Eq./L.	O.A. m.Eq. NaCl/kg. H_2O	(Cl.) m.Eq./L.
3	31.8	28.0	42.1	41.0	125.2	85.0	163.4	100.5	156.5	101.5
6	41.2	42.5	51.4	53.5	166.8		168.1	114.0	152.4	107.3
7*	34.3	34.5	43.7		165.2	100.0	183.0	100.0	160.9	95.0
8*	26.8	30.0			175.4	105.0	200.7		160.9	
9	48.8	44.0	74.6	70.5	162.1	94.5	169.6	103.0	163.5	98.5
10*	46.1	47.0	48.0	49.0	177.1		195.1		157.2	102.0
A			25.7		95.5					
B			12.7		94.2					
C			21.9		89.7		164.4			
D†			46.1		117.4		171.9			
E†			20.4		91.1		153.9			
Mean.	38.1		38.7		132.7		174.4		158.6	

* Gut not completely free of fecal material.

† 3 per cent body weight of H_2O given.

S1 = Fluid collected by stomach tube before anesthesia.

S2 = Fluid collected from stomach after anesthesia.

1 = Fluid collected from duodenum.

2 = Fluid collected from the beginning of the jejunum to end of the dye.

(2500 r.p.m.) for half an hour and the serum removed. The total osmotic activity, both of the gut samples and the serum, was determined on the Hill-Baldes vapor tension apparatus (14) in an atmosphere containing 5 per cent carbon dioxide and at a temperature of 37.5°C. The osmotic activity is reported in milliequivalents of sodium chloride per kilogram of water. (One milliequivalent is equal to 35.90 mm. mercury at 37°C.) In most cases chloride concentrations were determined by a semimicro modification of the Volhard method as described by Kolthoff (15).

A second series of animals was employed in the same way except that the animals were anesthetized with ether before the water was given.

Results and discussion. Table 1 shows the osmotic pressure and chloride

values for the series of animals that were anesthetized after the ten minute period of absorption. It is seen that in these animals the chloride concentrations and total osmotic pressures are approximately equivalent in the stomach samples, whereas the total osmotic pressures exceed predictions from the chloride values in the duodenum and jejunum. This is as would be expected due to the presence of bicarbonate and other anions in the secretions of the small intestine (10).

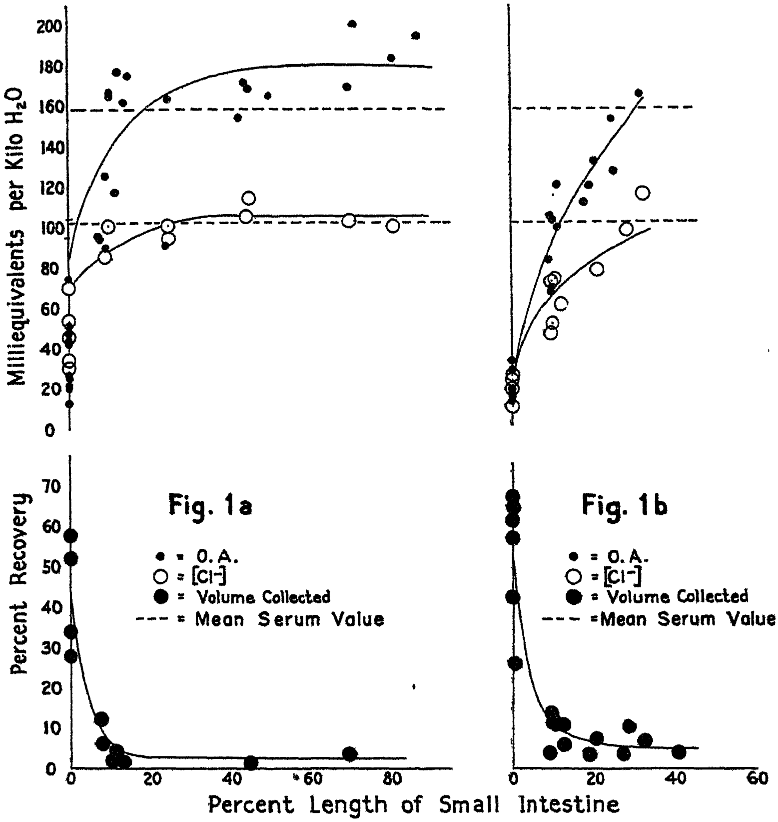

Fig. 1a. Osmotic activities, chloride concentrations, and volumes of fluid recovered from the gastrointestinal tracts of animals anesthetized after the ten minute period of absorption. "Per cent length of small intestine" is obtained by dividing the length of the segment in question by the length of the whole small intestine. The stomach samples are plotted as zero length. The total volume recovered from each animal is the sum of the volumes for each segment.

Fig. 1b. Values as in figure 1a for the animals etherized before the ten minute period of absorption.

Figure 1a relates these values to the portion of the small intestine from which the samples were withdrawn. The stomach samples are plotted as zero per cent intestinal length. The "per cent length of small intestine" for the duodenal and jejunal samples was determined by dividing the distance from the pyloric sphincter to the caudal clamp of the segment in question by the length of the whole small intestine (pyloric sphincter to cecum). The volume of the fluid recovered

(water unabsorbed) in each segment as a per cent of the water given is also plotted in figure 1a as a function of intestinal length. The volume recovered is assumed to be directly related to the quantity given since no fluid could be collected from the gastrointestinal tracts of animals that were not previously given water.

It is also noted (fig. 1a) that only ten minutes after administering a larger than "physiological" quantity of water, the duodenum sometimes, and the jejunum always contained at least isotonic fluid. In no case was the duodenal sample less than half isotonic.

Table 2 and figure 1b show the corresponding data for the animals which were given ether before the water was administered. Certain quantitative differences appear: 1. In this latter series of animals there was a greater per cent of fluid

TABLE 2

Osmotic activity and chloride concentrations in gastric, duodenal and jejunal fluids removed ten minutes after administration of water by stomach tube

(Rats anesthetized with ether before administration of water)

RAT NO.	S		1		2		SERUM	
	O.A. m.Eq. NaCl/kg. H_2O	(Cl.) m.Eq./L.	O.A. m.Eq. NaCl/kg. H_2O	(Cl.) m.Eq./L.	O.A. m.Eq. NaCl/kg. H_2O	(Cl.) m.Eq./L.	O.A. m.Eq. NaCl/kg. H_2O	(Cl.) m.Eq./L.
11	28.5	26.0	105.8	72.3	165.2	98.0		
12	15.9	10.0	67.3	46.8	116.4		154.7	
13	33.1	24.0	99.6	61.5			158.0	101.5
14	18.3	12.3	103.4	74.5	165.7	116.8	155.1	98.8
15	14.5	17.8			153.6		158.4	104.3
16	17.0	17.5	69.4	51.0	132.0	78.8	160.1	101.5
G	17.5		121.0		127.8			
H	13.9		81.5		110.0			
Mean..	19.8		92.6		141.3		157.3	

S = Fluid collected from the stomach.
1 = Fluid collected from the duodenum.
2 = Fluid from the beginning of the jejunum to the end of the dye.

recovered, especially from the duodenum and jejunum. 2. On the average the ingested water advanced over 26 per cent of the length of the small intestine, rather than over 43 per cent. 3. The osmotic activity values were on the whole lower. Statistical analysis (16) shows that the probability is slight that these differences in the mean values for osmotic activity readings result from errors in random sampling: P for the stomach samples is 0.004, for the duodenal samples 0.003, and for the jejunal samples 0.001.

Since intestinal motility is decreased under ether anesthesia (17) the smaller portion of intestine travelled may be explained in this manner. The resultant decrease in absorptive area (smaller length of intestine) must also be considered before concluding that ether actually decreases the rate of absorption from a given area of intestinal epithelium.

Since the intestinal secretions are considered approximately isotonic, the observation (table 1) that in each case the osmotic activity reading for the jejunal sample was in excess of that for the serum was unexpected and led to much speculation. The changes in osmotic activity as a function of time on the thermocouples (figs. 2 and 3) suggested bacterial activity as a causal agent, especially since the humidity, temperature, and gas mixture in the thermocouple chamber are such that bacterial growth would be facilitated. A real increase in osmotic activity might result from molecular breakdown, i.e., glucose to lactic acid or protein to amino acids, and a subsequent decrease in osmotic activity from the loss of carbon dioxide and ammonia formed on further breakdown.

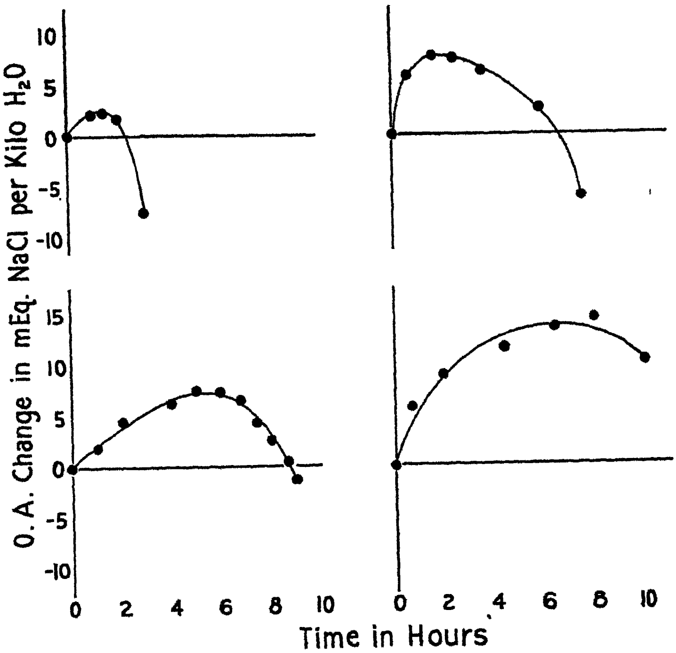

Fig. 2. Osmotic activity changes in four jejunal samples as a function of time after drop deposition. Note the variation in rate and extent of change.

Enough substrate to effect any of the observed changes has been calculated to be present (18). Due to the nature of the Hill-Baldes vapor tension method (19) an apparent increase in osmotic activity might result from heat production by bacterial growth. However, calculations (18) suggest that in these experiments heat production probably accounted for only a small part of the observed increases in osmotic activity.

That the observed osmotic activity changes in the jejunal samples over time are actually associated with bacterial activity is indicated by three types of experiments: 1. Both the increase and subsequent decrease in osmotic activity can be prevented by a bacterial poison, 0.5 per cent $HgCl_2$ (fig. 4). Moreover when the drop was poisoned during the course of the experiment, the rapid de-

crease in osmotic activity was stopped immediately. 2. Twenty-four hour pure cultures of lactobacillus acidophilus, chosen as the most abundant inhabitant of the upper small intestine of the rat (20), exhibited changes qualitatively similar

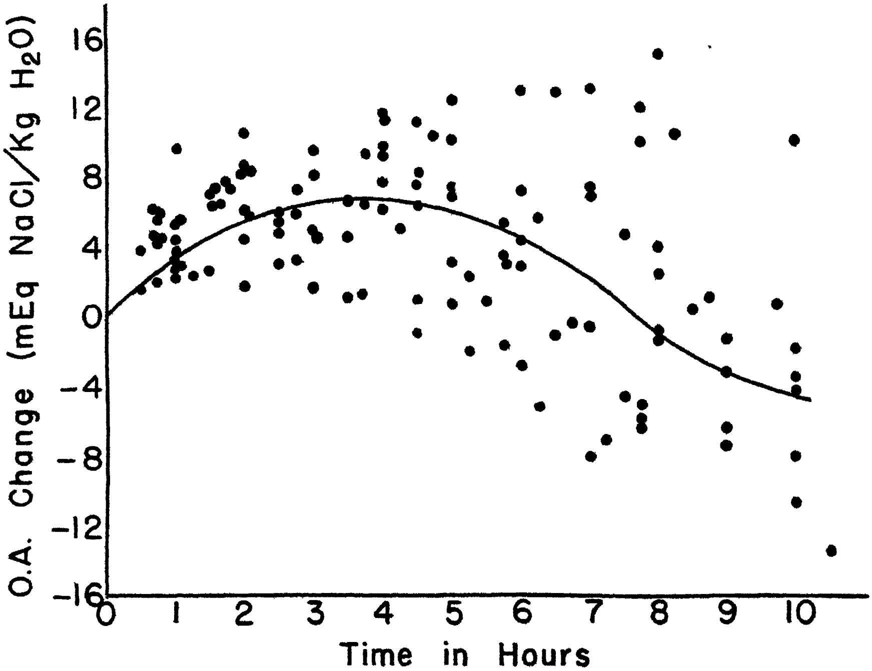

Fig. 3. Osmotic activity changes in eighteen jejunal samples as a function of time after drop deposition. The curve represents the calculated mean.

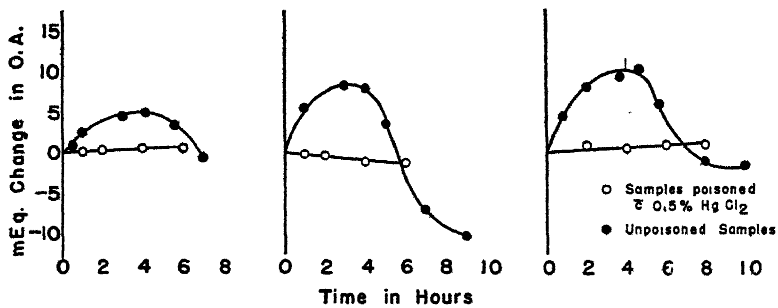

Fig. 4. Three experiments showing the effect of 0.5 per cent mercuric chloride upon the osmotic activity changes with time after drop deposition of jejunal fluids.

to those of the jejunal samples, whereas Zeitz filtrates behaved as the poisoned gut samples. 3. In two instances bacterial counts (extinction to dilution method, 21) showed that the actual number of organisms was increased in the

course of these experiments. Initial counts were 10,556 and 25,333 organisms per cc. At the peak in the osmotic activity increase in the former 9,138,889 organisms per cc. were present; shortly after the peak in the latter 77,900,000 organisms per cc. were present. The apparent hypertonicity of the jejunal samples initially observed, therefore, cannot be regarded as representative of osmotic relationships at the time of sample collection. Since the samples were ordinarily allowed to stand at room temperature for one to two hours before the osmotic pressure determinations were begun, the possibility of some bacterial activity during that time cannot be excluded. The results obtained with three samples poisoned with 0.5 per cent mercuric chloride immediately after collection (table 3) suggest that the marked hypertonicity of jejunal samples such as those listed for rats 7, 8, and 10 in table 1 is produced by bacterial activity subsequent to the time of collection. It is noted that these samples contained fecal material.

These observations on the time course of changes in osmotic activity of intestinal fluid samples point to the necessity of control of this factor in studies in which micro-organisms and organic materials are present.

TABLE 3

Osmotic activity of jejunal samples poisoned with 0.5 per cent $HgCl_2$ and corresponding serum samples

RAT NO.	OSMOTIC ACTIVITY IN m.Eq. NaCl/kg. H_2O	
	Jejunal samples*	Serum
26	151.3	159.3
28	163.1	160.7
29	173.3	159.7

* Corrected for the 0.5 per cent $HgCl_2$ added.

GENERAL SUMMARY AND CONCLUSIONS

1. Adult male white rats were fed by stomach tube 2 per cent of their body weight of water. After a ten minute period of absorption, samples were withdrawn from the stomach, duodenum, and jejunum. Two series of animals were employed: one in which the animals were anesthetized after the absorption period was completed, and one in which they were etherized before the water was administered.

2. All of the samples collected from the jejunum were approximately isotonic or slightly hypertonic, while those from the duodenum frequently were hypertonic. The stomach samples averaged 30 mEq. sodium chloride per kgm. of water. The volumes of fluid recovered agree roughly with those reported in the literature. Less concentrated fluid and larger volumes were recovered from the animals anesthetized before the water was given, than from those that were not etherized until later. The water given apparently progressed, in the latter series, farther down the gastrointestinal tract than it did in the former series.

3. Factors which might account for the apparent hypertonicity observed in

some of the jejunal samples were investigated. Evidence has been presented indicating that the apparent hypertonicity is associated with bacterial activity. Observations upon jejunal samples poisoned immediately after collection indicate that the fluid is probably approximately isotonic at the time of collection.

4. It is essential that osmotic activity measurements on fluids contaminated with micro-organisms and containing a metabolizable substrate be made virtually instantaneously upon collection or after prompt poisoning of enzymes and micro-organisms.

Acknowledgment is made to Dr. Maurice B. Visscher and Dr. Nathan Lifson or suggestions and assistance in this investigation.

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EFFECT OF ESTRONE AND DIETHYLSTILBESTROL ON GROWTH RATE OF RATS AND ON IODINE CONTENT OF THYROIDS[1]

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That there is a relationship between the ovaries and the thyroid in mammals has been observed and speculated upon for many years. The appearance of adolescent goiter and goiter during the climacteric has been noticed probably since the dawn of history. Until recently, however, the problem has been given relatively little attention from an experimental standpoint.

As to the effect of ovarian extracts on the thyroid, Elmer (2) admits controversies in the literature and states that further investigation is needed. Nakamura (14) was apparently the first one to study the effects of the hormones of the placenta and anterior lobe of the pituitary, and of the ovary on the iodine content of the thyroid. According to Hays (6) this report is so general, and the absence of quantitative data so obvious in the report that intelligent conclusions cannot be drawn. Petrova (16) found that in rabbits, under the influence of folliculin, the iodine content of the thyroid and of the blood decreased sharply as a result of a repression of the thyroid secretion. Kunde et al. (11) found no change in the thyroids of dogs receiving estrin for seventeen weeks. A repression of the activity of the rat thyroid by injections of menformone was observed histologically by Heyl et al. (7). Benazzi (1) reports that the administration of folliculin in large doses slows the functional rhythm of the mouse thyroid almost to the point of causing complete inhibition to the resorption of the colloid substance. The weight of the thyroid, according to Pincus and Werthessen (17), increased as a result of continued injection of theelin into young rats. The increase in weight of the thyroid was interpreted as meaning hypertrophy. Freudenberg and Clausen (3) observed a decrease in the weight of the thyroid of rats injected with theelin. Kreitmair and Seickmann (10) found that histological examination showed the thyroid glands of infantile rats injected with diethylstilbestrol to be resting and filled with colloid.

The inhibiting effect of the natural estrogens on growth in the rat has been observed by Spencer et al. (22) and Freudenberger et al. (3). The administration of diethylstilbestrol has been found by Noble (15), Gaarenstroom et al. (4), and Mellish et al. (13) to inhibit growth in the rat.

In addition to the estrogenic properties of diethylstilbestrol, workers (5, 10, 12,

[1] The data presented in this paper are taken from a Thesis submitted by Virgil L. Koenig to the Faculty of the University of Colorado in partial fulfillment for the degree, Doctor of Philosophy, in 1940.

[2] Now with Armour Laboratories, Armour and Company, Union Stock Yards, Chicago, Illinois.

20, 23) have observed toxic manifestations when diethylstilbestrol was administered in large doses to experimental animals. Among the toxic symptoms were kidney damage, liver damage, gastric ulcer, penumonia, and blood damage.

From a survey of the literature on the subject, it is not hard to agree with Elmer (2) that there is need for a more thorough investigation of the thyroid-ovarian relationship, and especially the effect of estrogens on the thyroid. It was the purpose of this investigation to study the effect of estrone and diethylstilbestrol on iodine content of the thyroid and on the rate of growth in rats.

Methods and procedures. The method used in this investigation for the estimation of iodine in the individual rat thyroid was that of Koenig and Gustavson (9). After the thyroids were removed from the animal they were placed in a little absolute alcohol until analyses could be made.

Thyroid tissue has great affinity for iodine, hence it was necessary to control the iodine intake of the experimental animals. This was accomplished by preparing a diet deficient in iodine and by administering known quantities of iodine as potassium iodide intraperitoneally when desired. Remington, Levine and von Kolnitz (19) found that a diet containing 47 to 72 parts per billion of iodine produced in rats a simple goiter which was alleviated by the addition daily of 1 to 2 micrograms of iodine as potassium iodide to the diet for each animal. More recently, Remington (18) and Sharpless (21) kept rats on iodine deficient diets and reported such diets to support normal growth and reproduction despite the fact that the thyroids were almost completely devoid of iodine and colloid.

In this investigation a diet proposed by Professor Wilgus of Colorado State College was used. Its composition expressed in parts per hundred was: ground yellow corn, 51.0; oat groats, 20.0; soybean oil meal (expeller process), 12.0; dried yeast residuals (brewers', Anheuser-Busch), 7.5; manganese sulfate, technical, 0.015; crude casein, 6.0; steamed bone meal, 2.5; sodium chloride, 1.0; and 1,000 D oil (activated animal pro vitamin D oil obtained from Dr. J. J. Waddell, Dupont de Nemours, Inc., diluted with cottonseed oil to a potency of 1,000 units per gram), 0.05. The iodine content of this diet by L. P. Ferris was found to be 21 parts per billion. Distilled water was given to the animals for drinking purposes. Estrone and diethylstilbestrol were used in the investigation. In order to compare the effect of estrone and diethylstilbestrol on the thyroid, it was necessary to give estrogenically equivalent doses. Ten rat units in each instance as found by Koenig et al. (8) were given subcutaneously each day.

Sixty female rats of the Yale strain about three weeks old and averaging 45 to 50 grams in weight were placed on the iodine-deficient diet and distilled water. These rats were weighed weekly. The rats were divided into three groups of twenty each. One group served as controls, the second group received 10 rat units (30 micrograms) of estrone dissolved in 0.2 ml. of olive oil subcutaneously each day, and the third group received 10 rat units (9.4 micrograms) of diethylstilbestrol dissolved in 0.2 ml. of olive oil subcutaneously each day. The site of injection was changed each day. At the end of twelve weeks the animals were sacrificed by injecting a 20 per cent solution of magnesium sulfate into the heart. The thyroids of a portion of the animals were removed and placed in Bouin's

fluid for histological study. The thyroids of the remaining, including a small piece of the trachea to insure complete removal of thyroid, were removed and placed in a small quantity of absolute ethyl alcohol until iodine determinations could be made.

Another group of sixty female rats of the Yale strain about three weeks old and averaging 40 to 50 grams in weight was placed on the same diet and distilled water. Each day these rats received intraperitoneally 2.44 micrograms of iodine as potassium iodide dissolved in 0.2 ml. of water. The weights of these rats were recorded weekly. These rats were divided into three groups of twenty each. One group served as controls, the second and third groups received ten units of estrone and diethylstilbestrol respectively as described above. At the end of twelve weeks, they were sacrificed in the same way as above. The thyroids from some of the animals of each group were used for histological study, while the thyroids of the rest of the animals were analyzed for iodine.

Results. The final average weights of the animals kept on the low iodine diet were as follows: for the controls, 218 grams, for estrone-treated, 184 grams, and for diethylstilbestrol-treated animals, 160 grams. The weekly weighings of the animals showed the same trend in their differences that was manifested in the final average weights.

Certain toxic manifestations were noticed in the animals treated with diethylstilbestrol. Nine out of twenty rats died before the end of twelve weeks. Upon autopsy, there were cheesy spots on the lungs, and the livers were spotted. These rats were emaciated, their body temperatures were low, and they had pronounced alopecia. Abscesses were found under the skin at the sites of injection. At the end of twelve weeks, when the rest of the animals were sacrificed, all the diethylstilbestrol animals had pronounced alopecia, and a number had ovarian cysts. In general, they appeared much worse than the other test animals. No striking symptoms were seen in the animals treated with estrone.

The results of the iodine determinations are given in the table. The average values show the estrone- and stilbestrol-treated animals to have a lower iodine content than the controls. The average iodine content of the thyroids of the controls is 2.20 micrograms, estrone-treated animals 1.45 micrograms, and diethylstilbestrol-treated animals 1.36 micrograms.

The average weights at the end of twelve weeks of the animals kept on the low-iodine diet supplemented with 2.44 micrograms of iodine each day in the form of potassium iodine were as follows: for the controls, 195 grams, for estrone, 159 grams, and for diethylstilbestrol, 154 grams. The final average weights reflect a trend which was characteristic of the weekly weighings throughout the experimental period.

The toxic effects of stilbestrol were not as striking as in the first series; however a few of the animals died before the experimental period was over. At the end of the twelve weeks when all the animals were sacrificed, the diethylstilbestrol animals had greatly enlarged pituitaries, spotted livers, and some of them had hyperemic uteri and ovarian cysts. Subcutaneous abscesses were found at the site of injection. Several suppurative tumors were seen in the abdominal cavities

of a number of the animals. All the diethylstilbestrol-treated animals were devoid of fat around the uteri and ovaries. Subcutaneous abscesses were seen in rats treated with estrone, and one or two ovarian cysts and spotted livers were observed, but the symptoms were not as pronounced as those of the diethylstilbestrol-treated animals.

The results of the iodine determinations are given in the table. The average value for the controls is 13.4 micrograms, for estrone-treated 18.5 micrograms, and for diethylstilbestrol-treated animals 11.4 micrograms.

TABLE 1

Iodine content of thyroids

RATS ON LOW-IODINE DIET			RATS ON LOW-IODINE DIET RECEIVING 2.44 μG. IODINE DAILY		
Control	Estrone (30 μg.)	Diethyl-stilbestrol (9.4 μg.)	Control	Estrone (30 μg.)	Diethyl-stilbestrol (9.4 μg.)
μg.	μg.	μg.	μg.	μg.	μg.
1.65	0.95	1.35	21.8	13.3	10.2
1.95	2.80	1.95	10.6	18.2	8.8
2.10	0.75	0.80	9.4	14.6	10.3
2.80	0.85	2.00	10.4	29.2	9.5
2.20	1.75	1.00	19.4	15.6	9.4
2.30	1.15	0.75	8.8	19.0	9.8
2.35	2.00	1.65	13.4	14.4	18.2
2.40	1.20		7.9	21.6	13.0
2.35	1.25		11.3	18.4	11.9
3.45	1.35		24.2	20.0	10.7
0.70	2.55		13.4	23.0	16.2
2.30	1.15		13.8	15.1	9.9
2.40	1.10		9.7		10.6
1.60					
Mean. .2.20	1.45	1.36	13.4	18.5	11.4
S.D. . . .0.53	0.62	0.48	4.9	4.3	2.8
S.E. . . .0.17	0.18	0.20	1.4	1.3	0.7
C.R. for control-estrone 3.09			C.R. for control-estrone 2.6		
C.R. for control-diethylstilbestrol . . . 3.24			C.R. for control-diethyl-stilbestrol . . . 1.2		

S.D. = Standard deviation.
S.E. = Standard error.

$$\text{C.R. (Critical ratio for significance)} = \frac{M_1 - M_2}{\sqrt{(\text{S.E.})_1^2 + (\text{S.E.})_2^2}}$$

DISCUSSION. Under the conditions of administration, diethylstilbestrol seemed to be more toxic than estrone. The toxic manifestations seemed to be much more evident on the low-iodine diet than on the low-iodine diet plus iodine. It is probably safe to assume that with inadequate iodine intake diethylstilbestrol was less rapidly metabolized and therefore was more toxic. Similar findings, but to a lesser degree were observed in the estrone-treated animals.

The administration of estrone and diethylstilbestrol retarded growth significantly in both groups of animals. In the group of animals kept on the low iodine

diet, diethylstilbestrol seemed to retard more than estrone—a difference of 58 grams and 34 grams, respectively, in the final average weights. With the animals on the low-iodine diet plus iodine, growth was retarded less by diethylstilbestrol although the final average weights were somewhat less than in the other group. For diethylstilbestrol and estrone, the weight decrease was 41 grams and 36 grams respectively. The adequate supply of iodine in the last group enabled metabolism to proceed at a more normal rate, thus accounting for a lower final average weight than in the group of animals without iodine added to the diet. The appetites of the animals kept on the iodine supplemented diet were better than those of the animals kept on the deficient diet.

There was a highly significant depression of the iodine content of the thyroids by diethylstilbestrol and estrone in the group of animals kept on the iodine deficient diet, inasmuch as the critical ratios (3.24 and 3.09, respectively) indicate a probability of less than one in one hundred that the difference is due to chance. The amount of depression seemed about equal in both cases. In the animals to which iodine was administered, considerable variation was experienced in the iodine content of the thyroids, and no statistical significance could be attached to the differences. Perhaps this variation is due to the great variation with which iodine is utilized by the thyroids of different animals as well as the variation in the size of the thyroid glands themselves. In any event, it is difficult to draw a definite conclusion about the effect of estrogens on the iodine content of the thyroids of animals receiving adequate iodine.

The authors are indebted to Dr. David Green of Merck and Company for the diethylstilbestrol, to Dr. Oliver Kamm of Parke, Davis and Company for the estrone, and to Prof. F. C. Koch for criticizing the manuscript.

SUMMARY

1. The administration of estrone and diethylstilbestrol over long periods of time retard significantly the growth of rats kept on diets deficient in iodine as well as when adequate iodine is available.

2. The toxic properties of diethylstilbestrol are more pronounced than estrone when given in estrogenically equivalent doses. Toxic manifestations are more accentuated in animals on an iodine deficient diet than on a normal diet.

3. Both diethylstilbestrol and estrone cause significant depression of the iodine content of the thyroids of rats kept on an iodine deficient diet.

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NITROGEN BALANCE AND PLASMA PROTEIN REGENERATION IN HYPOPROTEINEMIC DOGS[1]

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The influence of amino acids and proteins on plasma protein regeneration has been extensively studied in hypoproteinemic dogs by several methods. Weech (1), for example, depleted dogs by feeding a low protein diet after which the protein intake was increased and plasma albumin regeneration was measured. Whipple and co-workers (2–4) used the plasmapheresis technique to deplete the animals and to measure the plasma protein regenerations. Melnick, Cowgill and Burack (5) used a similar technique, but fed "equal absolute amounts of the respective proteins above the minimum of each required to establish nitrogen equilibrium." This method assumes that the nitrogen excretion and the utilization of proteins are the same in both normal and depleted dogs.

There is a need for a method for the study of plasma protein regeneration in which the regeneration of the various plasma proteins can be correlated with the source of dietary nitrogen and the nitrogen balance produced. The method described below was developed so that the nitrogen excretion before, during and after regeneration could be measured, utilization of the protein calculated, regeneration of plasma proteins determined and nitrogen balances established. The dogs were depleted by a combination of protein-free feeding and plasmapheresis; the latter performed during the depletion period in order to hasten the protein depletion process.

METHODS. Normal dogs were starved for three days and placed on the protein-free diet used by Allison and Anderson (6). The diet consisted of 21.6 per cent sucrose, 19.4 per cent dextrin, 32.9 per cent dextrose, 21.8 per cent lard, 1.8 per cent Wesson's modified Osborne-Mendel salt mixture (7) and 2.5 per cent agar. The following vitamins were included in the diet; thiamin, riboflavin, nicotinic acid, calcium pantothenate, pyridoxine, choline, 2-methyl-1-napthoquinone and vitamins A and D. The dogs received daily 70 calories of food per kilogram body weight.

After six to ten days of partial depletion of protein reserves by protein-free feeding, plasmapheresis was performed daily for two to five days. During plasmapheresis one quarter of the blood volume of the dog was removed each day and an equivalent amount of washed red blood cells, suspended in physi-

[1] The data presented are taken from a thesis presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Physiology and Biochemistry, Rutgers University, New Brunswick, New Jersey.

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ological saline, reinjected immediately. This was followed by protein-free feeding alone until the plasma protein concentration of the dog remained at a relatively constant value between 4.0 to 4.5 grams of protein per 100 ml. of plasma for five days.

Proteins and protein hydrolysates were fed orally or injected intravenously into the dogs for another period of five days. The protein was included in the diet by replacing equivalent amounts of calories from the carbohydrates. Following the five day protein-feeding period, the dogs were again fed the protein-free diet until the plasma proteins returned to the previous concentrations on the protein-free diet.

Five day nitrogen balances were determined during the experiment. A control balance was carried out during the first five days of protein-free feeding. A second balance was determined just before the feeding period (fore-period). This balance measured the "endogenous" nitrogen excretion of the dog in the depleted state. Another balance was carried out during the five days of protein feeding (protein-intake period). A fourth nitrogen balance was determined immediately after the end of the protein-intake periods (after-period). The dogs were catheterized before and after each balance period. The urine was acidified with H_2SO_4 and collected under toluene. Carmine red capsules were used to mark the feces which were collected in friction-top cans. The urine and feces for each period were pooled and kept in the ice box until analyzed.

Daily 10 ml. blood samples were collected from the jugular vein, using heparin as an anticoagulant for plasma protein determinations. Plasma volume determinations were carried out twenty-four hours after the last day of feeding in each period using the T-1824 dye method of Gregersen and Stewart (8). Blood samples were taken at 9:00 a.m. and the dogs were fed at 1:00 p.m. Catheterization was performed just before feeding.

The plasma was analyzed for total protein, albumin, globulin and non-protein nitrogen. The chemical fractionation of the plasma was carried out by the method of Howe (9) as modified by Robinson, Price and Hogden (10) which is based upon the precipitation of globulin by 22.5 per cent Na_2SO_4 at 37°C. The total nitrogen of the filtrates and diluted plasma as well as of the urine and feces was determined by the Pregl micro-Kjeldahl method. After correcting for non-protein nitrogen the remaining nitrogen of the plasma was converted into protein by the factor 6.25. Globulin was determined by subtracting the albumin from the total plasma protein.

Results. The effect of protein depletion upon the nitrogen excretion on a protein-free diet ($N.E._o$) is recorded in table 1. The urinary nitrogen excretion in the control period was 0.193 gram of nitrogen per kilogram body weight per day. After depletion the average urinary nitrogen dropped to 0.106 gram of nitrogen per kilogram per day. The extent of depletion is reflected in the drop in plasma protein concentration from a control of 5.99 grams per cent to 4.51 grams per cent in the depleted state. Dogs with a lowered urinary nitrogen excretion and plasma protein concentration were used to study the plasma protein regeneration.

Figure 1 (dog 28-2) illustrates the results of regeneration of plasma proteins in which bovine serum protein was the source of dietary nitrogen. The dog received orally 0.35 gram of protein nitrogen per kilogram body weight. The animal was put into a nitrogen balance of +0.14 gram of nitrogen per kilogram per day (table 2). The average plasma protein concentration during the fore-period was 4.34 grams per cent. After the fourth day of protein feeding the plasma protein concentration showed a definite rise, reaching a maximum value of 5.00 grams per cent two days later. The concentration remained elevated for five days and then dropped quickly to the fore-period level.

The concentration of plasma albumin and globulin for dog 28-2 during the experiment are illustrated below the curve for total protein in figure 1. There was no change in globulin concentration during the experiment, remaining at an average value of 1.94 grams per cent. All the rise in plasma protein concentration was the result of an increase in plasma albumin. The albumin concentration increased from 2.40 grams per cent in the fore-period to a maximum of 3.20 grams per cent. Since the animal was depleted primarily in the albumin fraction and less in the globulin, the increase in plasma protein concentration during regeneration was the result of a return toward normal of that protein in which the dog was most depleted.

TABLE 1

Plasma protein concentration and urinary nitrogen excretion on a protein free diet (N.E.$_o$) before and after depletion

DOG. NO.	PLASMA PROTEINS		URINARY NITROGEN EXCRETION ON PROTEIN FREE DIET (N.E.$_o$)	
	Control	Depleted	Control	Depleted
	gms. %	*gms. %*	*gms./kilo/day*	*gms./kilo/day*
32-1	5.07	4.27	0.231	0.138
28-1	6.00	4.66	0.197	0.079
31-1	5.94	4.70	0.157	0.118
30-2	6.20	4.46	0.178	0.113
33-1	6.40	4.23	0.232	0.109
27-1	6.96	4.59	0.170	0.096
30-1	5.40	4.66	0.184	0.092
Ave.	5.99	4.51	0.193	0.106

The plasma volume determinations on dog 28-2 in figure 1 were made to correct for any changes in protein concentration which could result from fluid shifts alone. Since there were no marked changes in plasma volume during this experiment the curves drawn through the points of concentration give a true picture of shifts in total circulating proteins.

Dog 31-1 received orally 0.20 gram of nitrogen per kilogram per day of bovine serum protein. The nitrogen balance produced was +0.05 (table 2) instead of +0.14 gram nitrogen per kilo per day in the previous experiment. Again the rise in plasma protein concentration came after several days of protein feeding

but the return to the depleted state was more rapid (fig. 1). Thus, with less nitrogen available for growth there was a smaller increase in plasma protein concentration and a more rapid return to the depleted state. As in the previous experiment the dog was depleted primarily in plasma albumin and regeneration was restricted to this plasma protein. Plasma volume changes were not marked.

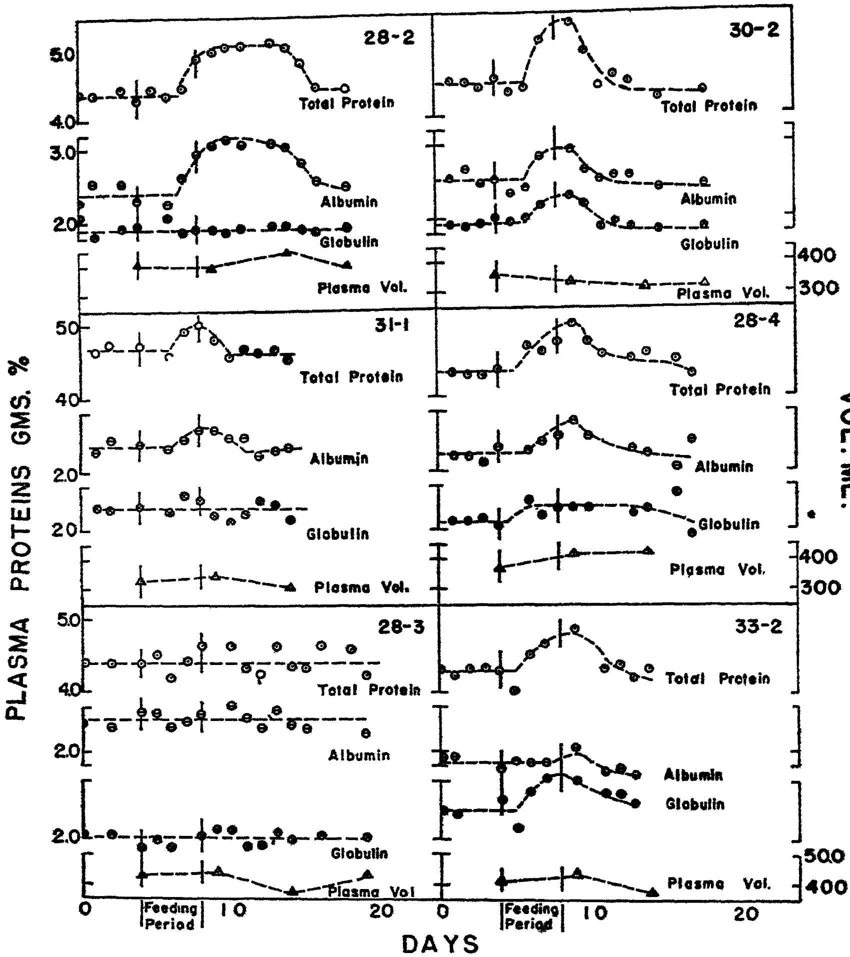

Fig. 1. The effect of protein feeding upon plasma protein regeneration in six depleted dogs. Dogs 28-2, 33-1 and 28-3 received respectively 0.35, 0.20, and 0.09 gram of bovine serum protein nitrogen per kilogram per day during the feeding period. Dogs 30-2 and 28-4 received 0.36 gram of casein nitrogen per kilogram per day and dog 33-2 received intravenously 0.37 gram of amigen nitrogen per kilogram per day.

Dog 28-3 was fed orally 0.09 gram of nitrogen per kilogram per day of bovine serum protein during the feeding period. The dog was in nitrogen equilibrium with a nitrogen balance of 0.00 (table 2). As there was no nitrogen available for growth the plasma protein showed no indication of sustained regeneration (fig. 1).

These data demonstrate that a positive nitrogen balance is necessary for plasma protein regeneration and that the degree and duration of regeneration is dependent upon the magnitude of this positive balance.

Casein was fed orally in another experiment on a depleted dog (dog 30-2). This dog was fed 0.36 gram of nitrogen per kilogram per day and maintained a nitrogen balance of +0.11 during the feeding period (table 2). This dog like the others was taken from the constant state of depletion through a regeneration

TABLE 2

Nitrogen intake, urinary nitrogen, fecal nitrogen and nitrogen balances for six depleted dogs before, during and after protein feeding

The bovine serum protein and casein were fed orally. The amigen was fed intravenously. All the periods were five days.

DOG NO.	PROTEIN SOURCE	PERIOD	NITROGEN INTAKE	NITROGEN URINARY	NITROGEN FECAL	NITROGEN BALANCE
			gms./kilo/day	*gms./kilo/day*	*gms./kilo/day*	*gms./kilo/day*
28-2	Bovine serum	Fore	0	0.09	0.03	−0.12
		Feeding	0.35	0.16	0.05	+0.14
		After	0	0.08	0.02	−0.10
31-1	Bovine serum	Fore	0	0.12	0.03	−0.15
		Feeding	0.20	0.12	0.03	+0.05
		After	0	0.09	0.02	−0.11
28-3	Bovine serum	Fore	0	0.08	0.02	−0.10
		Feeding	0.09	0.07	0.02	0.00
		After	0	0.07	0.02	−0,09
30-2	Casein	Fore	0	0.11	0.04	−0.15
		Feeding	0.36	0.21	0.04	+0.11
		After	0	0.09	0.03	−0.12
28-4	Casein	Fore	0	0.07	0.02	−0.09
		Feeding	0.36	0.15	0.03	+0.18
		After	0	0.06	0.02	−0.08
33-2	Amigen*	Fore	0	0.10	0.03	−0.13
		Injection	0.37	0.29	0.03	+0.05
		After	0	0.06	0.02	−0.08

* Injected intravenously.

period and back to the depleted state (fig. 1). The same lag period between the initial protein feeding and the regeneration of plasma protein is evident in these data. There is one difference between this experiment and those described for bovine serum protein. The regeneration is not limited to albumin, but includes the globulin fraction. A duplicate experiment on casein produced the same stimulation of globulin regeneration (dog 28-4). Thus there may be differences between proteins in their ability to stimulate regeneration of the various fractions of plasma proteins.

This method of study may be applied to intravenously fed protein hydrolysates as well as orally fed protein. In table 2 are the results on dog 33-2 from an experiment in which a casein hydrolysate "amigen"[2] was administered intravenously. The dog received 0.37 gram of nitrogen per kilogram per day for five days producing a nitrogen balance of +0.05 gram of nitrogen per kilo per day. The plasma proteins (fig. 1) increased from a pre-injection level of 4.3 grams per cent to a maximum of 4.7 grams per cent. The rise in plasma protein was confined primarily to the globulin fraction where the concentration rose from an average of 2.3 grams per cent in the foreperiod to the maximum of 2.7 grams per cent. The plasma volume showed no appreciable changes.

The areas under the curves showing the change in concentration of plasma proteins from the depleted state through regeneration and back again to the

TABLE 3

The plasma albumin and globulin concentrations in protein depleted dogs, the source of protein fed for a period of five days to the depleted dogs, the nitrogen balances obtained, and regeneration areas

The regeneration area was taken to be the area under curves (grams per cent of plasma protein vs. days) rising above the plasma protein concentration in the depleted state (see fig. 1).

DEPLETED STATE		PROTEIN SOURCE	NITROGEN BALANCE	REGENERATION AREA (Δ PROTEIN × DAYS)		
Albumin	Globulin			Total protein	Albumin	Globulin
gms. %	*gms. %*		*gms./kilo/day*			
2.4	2.0	Bovine serum	0	0	0	0
2.4	2.3	Bovine serum	+0.05	0.7	0.7	0
2.4	1.9	Bovine serum	+0.14	5.1	5.1	0
1.6	2.6	Bovine serum	+0.15	3.6	4.7	−1.1
2.5	2.0	Casein	+0.11	2.6	1.3	1.3
2.2	2.1	Casein	+0.18	3.3	1.4	1.9
1.9	2.4	Amigen*	+0.05	1.8	0.2	1.6

* Intravenous.

depleted state may be used to measure the degree of plasma protein regeneration. The regeneration areas for bovine serum protein, casein and amigen are recorded in table 3. The regeneration area of total protein and albumin increased with increasing positive nitrogen balances when bovine serum protein was fed at different levels. When casein was fed orally the regeneration areas of both albumin and globulin increased. The intravenous feeding of the casein-hydrolysate "amigen," under the experimental conditions used, caused an increase primarily in globulin.

The biological values of the proteins fed orally and intravenously to depleted dogs are tabulated in table 4. The values were calculated by the method of Allison and Anderson (6) assuming that their equation for biological values in

[2] "Amigen" is the trade name for the casein hydrolysate prepared by Mead Johnson and Company, Evansville, Indiana.

normal dogs in negative or low positive balance would be applicable for protein-depleted dogs. The biological values for bovine serum protein and casein fed orally was 0.98 and 0.97 respectively. Amigen which was administered intravenously had a biological value of 0.75 and three different pancreatic hydrolysates, also given intravenously, showed values of 0.42, 0.37 and 0.00.

Discussion. Protein depletion in dogs causes a corresponding drop in urinary excretion of nitrogen on a protein-free diet. This decrease in "endogenous" nitrogen has been reported in humans by Deuel et al. (11) and shown to be primarily due to a decrease in urea nitrogen. The depleted dog tends either to conserve body nitrogen or the protein catabolism decreases as a result of a smaller amount of protein available for this process. The decrease in urinary nitrogen during protein depletion may be used to indicate the degree of hypoproteinemia.

The amount of plasma protein regeneration which occurs on feeding proteins to protein-depleted dogs seems to be dependent upon the degree of positive nitrogen balance. Dogs receiving protein either orally or intravenously in amounts which keep them in negative or zero nitrogen balance do not regenerate plasma protein. Animals which are fed enough protein to put them in positive nitrogen balance exhibit definite rises in plasma protein concentration. The regeneration areas are roughly proportional to the extent of positive balance. Definite lag periods of from two to three days after the initial protein feeding occur before the plasma protein concentration increases above the fore-period levels. This lag in regeneration may be the result of the protein being used for the formation of proteins in the body other than plasma protein. The amount of nitrogen used for the formation of plasma proteins is less than 10 per cent of the nitrogen that the dog utilizes. The lag period is not due to a change in daily utilization of the protein. Later experiments in which the dogs were catheterized each day showed that the urinary nitrogen excretion was constant throughout the feeding period. Holman, Mahoney and Whipple (2) reported similar lag periods in their regeneration experiments.

TABLE 4

Absorbed nitrogen at equilibrium and biological values for depleted dogs

The bovine serum protein and casein were fed orally. The amigen and the three different pancreatic hydrolysates were fed intravenously.

DOG NO.	PROTEIN SOURCE	ABSORBED NITROGEN AT EQUILIBRIUM	B.V.
		gms./kilo/day	
31-1	Bovine serum	0.142	0.98
32-1	Casein	0.165	0.97
21-5	Amigen	0.191	0.75
21-2	Pancreatic hydrolysate (A)	0.307	0.42
30-1	Pancreatic hydrolysate (B)	0.396	0.37
27-1	Pancreatic hydrolysate (C)		0.00

The type of plasma protein regenerated by depleted dogs appears to be specific

for the individual proteins fed. Under the experimental conditions used beef serum protein regenerated primarily albumin, casein regenerated both albumin and globulin and amigen given intravenously favored globulin formation. How much of the regeneration of globulin can be attributed to something specific in casein or casein hydrolysates is open to further experimentation.

Although casein and bovine serum protein showed differences in the type of plasma protein regenerated, the biological values were almost equal. The biological values of casein and bovine serum protein in normal dogs also are approximately the same, but lower than those obtained on the depleted dog. Allison and Anderson (6) report a biological value for casein of 0.81. The values for casein and beef serum protein in the depleted dogs are 0.97 and 0.98 respectively. Too much significance cannot be attached, however, to these values until more complete data are available to show the change of biological value with increasing nitrogen intake in depleted dogs. If the degree of positive nitrogen balance determines the extent of plasma protein regeneration, the determination of the utilization of various proteins in the depleted animal would be an index to the amount of plasma protein the dog would regenerate. Further investigation is necessary to verify this possibility.

SUMMARY

1. A method is described for the quantitative study of plasma protein regeneration in dogs which takes into consideration the biological value of the protein, nitrogen balance and specific effects on plasma albumin and globulin formation.
2. The urinary nitrogen excretion on a protein-free diet is decreased in protein depleted dogs.
3. Under the experimental conditions used beef serum protein fed orally causes regeneration of albumin, casein favors both albumin and globulin regeneration and the casein hydrolysate "amigen" administered intravenously stimulates globulin formation.
4. Plasma protein regeneration in hypoproteinemic dogs increases with increasing positive nitrogen balance.
5. The biological values of orally fed bovine serum and casein protein and intravenously fed casein hydrolysate and three pancreatic hydrolysates are presented.

The author wishes to express his indebtedness to Dr. James B. Allison and Dr. William H. Cole of the Department of Physiology and Biochemistry, Rutgers University, for their interest, advice and encouragement during the course of this investigation.

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RESULTS. Four types of experiments were performed on each of three trained male subjects. These observers were students ranging in age from 15 to 17 years, who had no physical or ocular abnormalities. All experiments were carried out in the morning, the subjects having had no food since the previous evening.

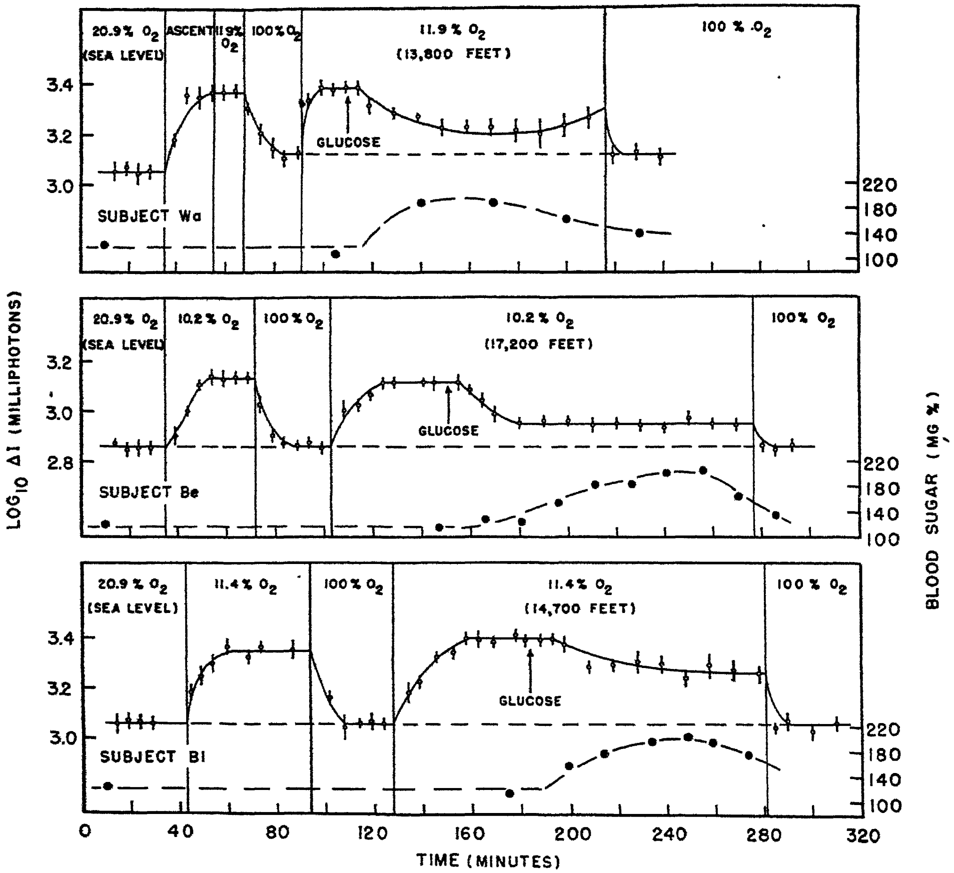

Fig. 1. The reversal of the effects of anoxia by glucose. The results of experiments on three subjects are represented. Differential intensity thresholds are plotted against time. Each point is the mean of ten measurements. The vertical line through each point denotes plus and minus the standard deviation. A rise in the curve signifies impairment of visual sensitivity.

After the administration of glucose, the curve drops while the blood sugar (broken curve) rises. Subject Wa shows a secondary rise coincident with the subsequent fall of the blood sugar curve.

A. *The reversal of the effects of anoxia by glucose.* The results and procedure are indicated in figure 1, where visual thresholds are plotted against time. Each point represents the mean of ten measurements; the length of the vertical line through each point represents plus and minus the standard deviation (in logarithmic units). The standard deviations were determined by the expression $\sigma = \sqrt{\Sigma d^2/n-1}$ where d is the deviation of each measurement ($\log_{10} \Delta I$) from the

mean, and n is the number of measurements. The standard deviations are a measure of the variability of each group of measurements.

After several groups of measurements had been made in normal air, containing 20.9 per cent oxygen, the oxygen tension was reduced to the indicated value (fig. 1). The notation "ascent" denotes a gradual reduction in the oxygen percentage; otherwise the change in the composition of the inspired air was abrupt. Readings were taken until a constant threshold was reached. In all cases, an elevation of the threshold was observed. Oxygen was then administered in order to demonstrate the reversibility of this change, and the rapidity with which it could be brought about. In general, the same level was reached with oxygen as at the beginning of the experiment in ordinary air.

The subject was re-exposed to the low oxygen tension and observations were again made until constant readings were obtained. The threshold rise was approximately equal to that previously observed.

The subject then drank a solution containing 50 grams of glucose in 200 ml. of water. The visual thresholds began to decrease toward the normal value about ten minutes after ingestion of glucose and continued to decrease as the blood sugar concentration rose. The data for subject Wa show the clearest parallelism between blood sugar and visual sensitivity. In this case, the greatest improvement in visual sensitivity occurred about one hour after ingestion of glucose, when the blood sugar reached its maximum. When the blood sugar level subsequently decreased, the threshold again began to rise. In one case, subject Be, the drop in threshold was initially more rapid than the rise in blood sugar, but then it levelled off while the blood sugar continued to rise. This suggests the possibility that beyond a certain elevation of the blood sugar level, any further rise may be comparatively ineffectual.

Neither subject Be nor Bl showed a rise in threshold when the blood sugar began to decline, but the observations were not carried out for a sufficiently long period to yield significant conclusions regarding this point. It may be noted that in subsequent experiments reported below, in which measurements were continued for a longer period after glucose administration, all three subjects showed a rise in threshold when the blood sugar level declined.

As a final step, oxygen was again given to the subject. This resulted in a return of the threshold to its earlier lowest level.

The statistical significance of the changes observed after glucose may be judged from the relation between the magnitude of these changes, and of the standard deviations (denoted by the vertical lines through the points). Since the observed changes are in all cases much larger than the standard deviations, they are statistically significant.[3]

[3] More precisely, a change or difference is statistically significant when it is at least three times as large as its standard error. The standard error of the difference between any one point denoting a mean, and a level established by a number of other such points is essentially equal to, or but slightly larger than, the standard error of the point itself. The latter is determined by dividing the standard deviation of the point by the square root of the number of measurements on which the point is based ($\sigma_m = \sigma/\sqrt{n}$). In this case $n = 10$. Thus the standard deviation σ is over three times the standard error. Hence any difference greater than the standard deviation of a point is statistically significant.

B. *The prevention of the effects of anoxia by the administration of glucose.* This group of experiments differed from those described above, in that the glucose was administered one-half hour before re-exposure to the low oxygen tension, instead of during exposure. In this way, the blood sugar was already at a high level at the time when the subject was deprived of a normal oxygen supply. The resultant threshold rise could then be compared with that which occurred during anoxia prior to glucose administration.

The results of this series are indicated in figure 2. Broken horizontal lines denote the level of the thresholds during oxygen deprivation before glucose administration. It is obvious that the rise in threshold upon re-exposure to the same oxygen tension one-half hour after ingestion of 50 grams of glucose in 200 ml. of water is not nearly so great. The inverse relationship between the visual thresholds and the blood sugar level subsequent to the first ten minutes of re-exposure (this period being required for the effect of the anoxia to become apparent) is clearly demonstrated by the graphs.

The data for subjects Bl and Wa in which the maximum blood sugar level occurred at about this time show that the thresholds subsequently underwent a gradual rise as the blood sugar declined. Subject Be showed a further rise in blood sugar for about 30 minutes, accompanied by a slight lowering of his thresholds. When the blood sugar subsequently decreased, there was a coincident rise in the thresholds. Finally, oxygen restored the thresholds to their original values.

The statistical significance of the changes is estimated as in figure 1. Since the standard deviations of the measurements are much smaller than the effect of the glucose, this effect is considered to be statistically significant.

C. *Control experiments with saccharin during anoxia.* The procedure was identical with that used in the first group of experiments, (A), with the exception that a saccharin solution (2 to 3 grains in 200 ml. of water) was substituted for glucose. The subjects were able to detect some difference in taste between these solutions but were told that different types of sugars were being studied. They were not aware that these were control experiments.

The absence of any change in visual sensitivity after saccharin administration during anoxia is demonstrated in figure 3. The thresholds remained constant during the 70 to 90 minutes of anoxia after saccharin. When, finally, oxygen was administered, the thresholds were restored to their original values.

The lack of statistical significance of the small fluctuations in threshold observed after saccharin is indicated by the fact that in all cases these deviations of the points from the curve are smaller than the standard deviations of the points.

D. *The effect of glucose in the presence of a normal oxygen supply.* After the threshold level in normal air had been determined, fifty grams of glucose were administered orally. Visual measurements were continued for one-half hour, by which time there was a marked elevation of the blood sugar level to 149, 160 and 190 mgm. per cent for subjects Be, Bl and Wa, respectively. However, no change in visual sensitivity was observed. In a previous study McFarland and

Forbes found that the dark adaptation of fasting individuals was improved after a breakfast. It was suggested that this effect was due to the rise in blood sugar. The present results indicate that a rise in blood sugar above normal fasting levels

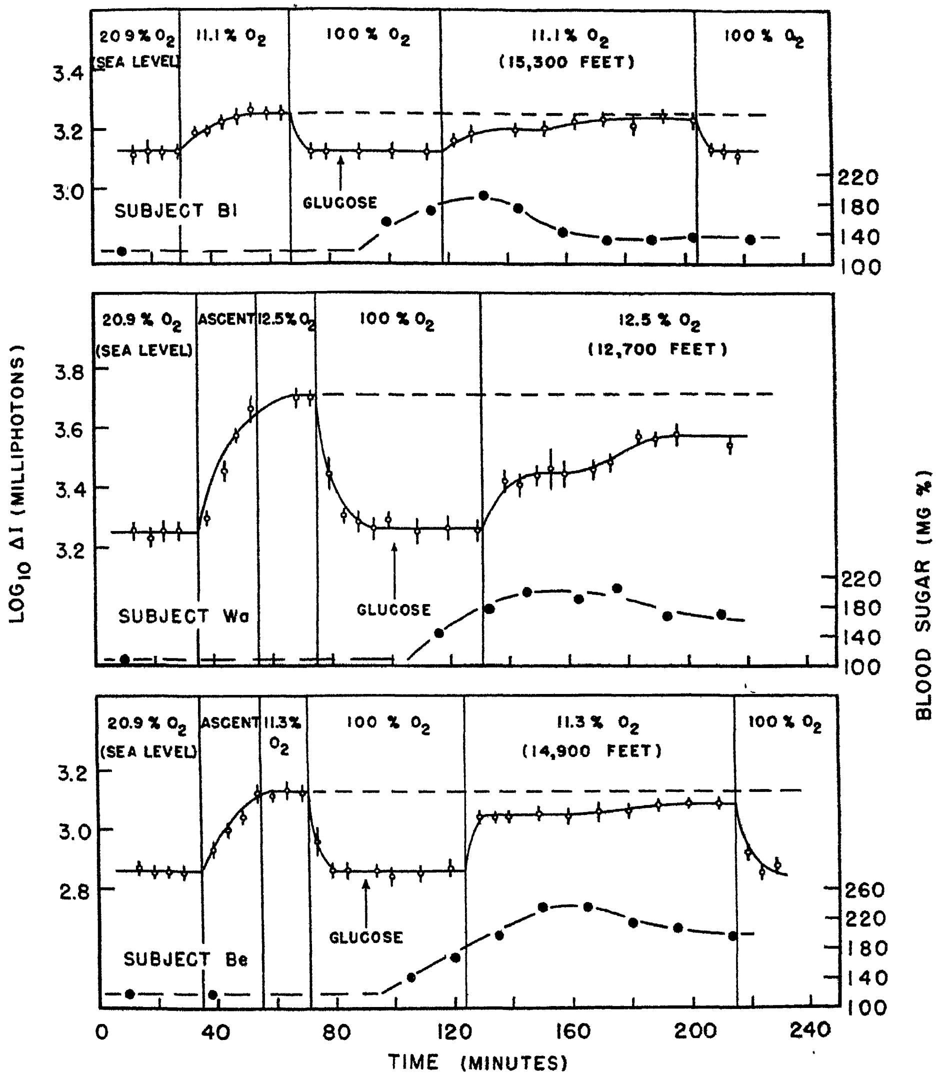

Fig. 2. The prevention of the effects of anoxia by glucose. The rise in thresholds upon exposure to simulated high altitudes one-half hour after ingestion of glucose is much less than that without glucose. As the blood sugar drops, the thresholds of all three subjects rise.

improves visual sensitivity only if this has first been impaired by anoxia. Otherwise, it does not cause an improvement above the initial level of sensitivity.

Discussion. A. *The effect of glucose on physiological altitude.* These studies

indicate that the ingestion of glucose at high altitudes results in a diminution of the effect of oxygen-lack. Thus glucose decreases the effective or "physiological" altitude while the individual remains at a constant actual altitude or oxygen tension. The quantitative effect of glucose on the physiological altitude of the

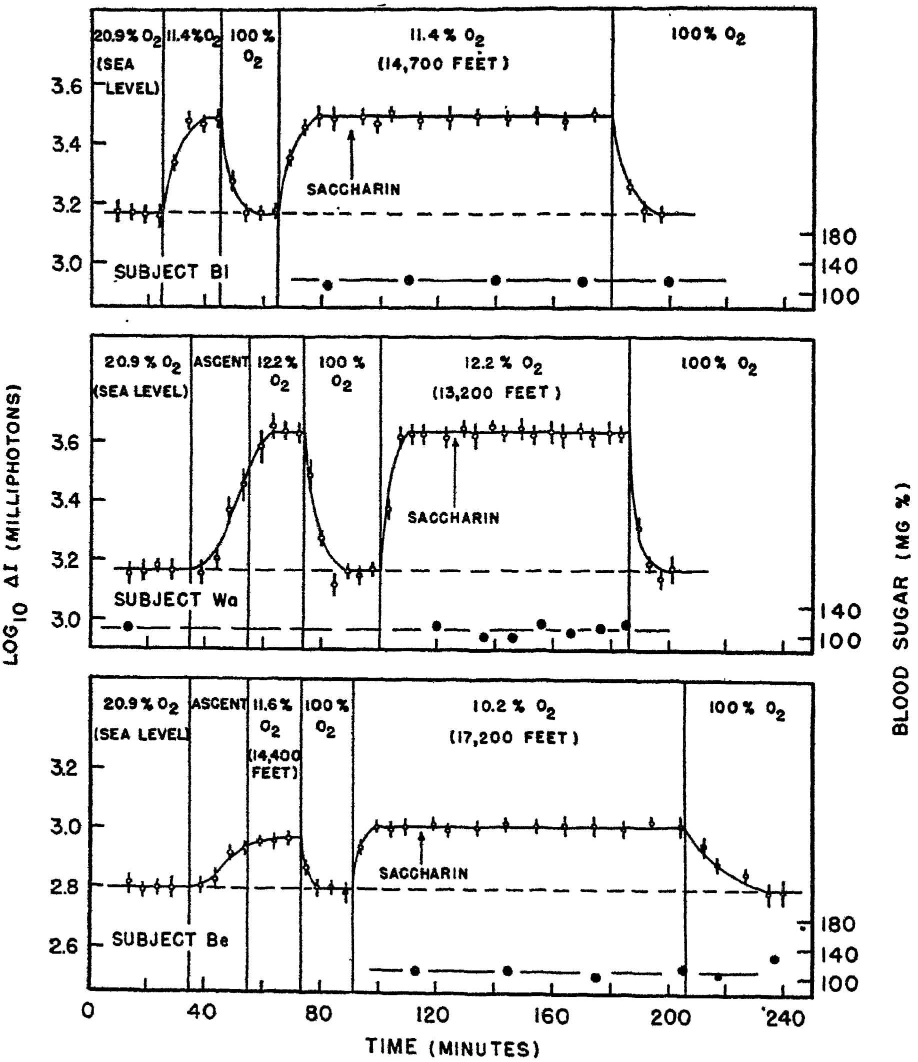

Fig. 3. Control experiments with saccharin during anoxia. The administration of a saccharin solution at simulated high altitudes has no effect on visual thresholds.

subjects during the present experiments was determined in the following manner. The visual mechanism of each subject was "calibrated" as a device for measuring altitude-effects by determining its response to progressive degrees of anoxia. Curves showing the relationship between altitude and the associated rise in

threshold for each subject are shown in figure 4A. By means of these curves, given threshold elevations may be equated to corresponding altitudes.

The sensitivity of this visual function to anoxia varies from day to day, although the form of the curve for a given subject remains the same. This variation has to be taken into consideration when comparing experiments done on different days. To do this it was necessary to calibrate the sensitivity of the subject to anoxia on each day an experiment was done. By comparison of this degree of sensitivity with that on a standard day, a factor was determined by which to correct all threshold changes to a basis of constant sensitivity. For

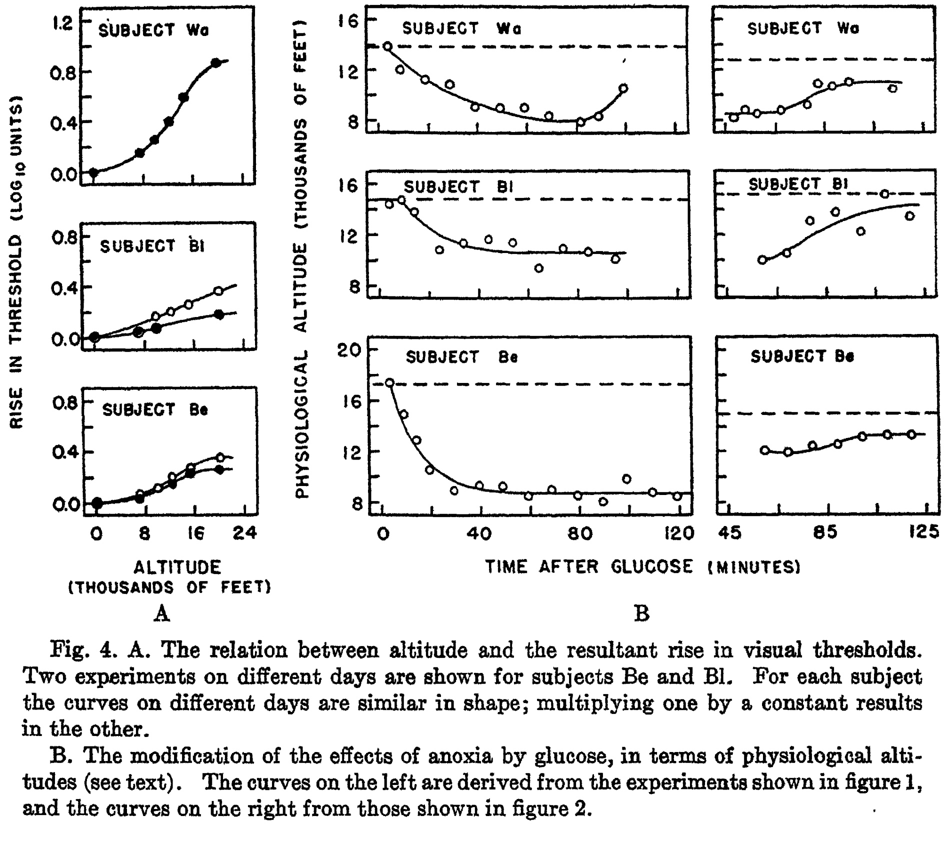

Fig. 4. A. The relation between altitude and the resultant rise in visual thresholds. Two experiments on different days are shown for subjects Be and Bl. For each subject the curves on different days are similar in shape; multiplying one by a constant results in the other.

B. The modification of the effects of anoxia by glucose, in terms of physiological altitudes (see text). The curves on the left are derived from the experiments shown in figure 1, and the curves on the right from those shown in figure 2.

subjects Be and Bl the upper curves in figure 4A were arbitrarily taken as the standards, and the experimental threshold changes presented above were corrected so as to be comparable to these. This was done by comparing the rise in threshold at the experimental altitude, prior to glucose, with the rise in threshold on the standard curve at the same altitude. The factor by which the former must be multiplied to equal the latter was computed. The rises in threshold (above normal) after glucose were then also multiplied by this same factor, yielding corrected values comparable with the standard. By employing the curves in figure 4A, the altitude corresponding to a given corrected rise in thres-

hold at subsequent times during the same experiment was determined. This was taken as the physiological altitude.

The physiological altitudes corresponding to the points after glucose in figure 1 are shown in the left-hand graphs of figure 4B. The broken line represents the experimental altitude in each case. This manner of presentation shows more clearly the magnitude of the effect of glucose, although the shapes of the curves are similar to those in figure 1. Thus, glucose resulted in a lowering of the physiological altitude from 13,800 feet to about 8,000 feet in the case of subject Wa; from 14,700 feet to about 11,000 feet in the case of subject Bl; and from 17,200 feet to about 9,000 feet in the case of subject Be. These decreases represent 42 per cent, 25 per cent and 48 per cent of the true altitudes in the three cases. Similarly, the physiological altitudes corresponding to the points in figure 2 (following the first 10 to 15 min. after re-exposure to anoxia, to permit time for the latter to manifest its effects) are shown in the right-hand graphs of figure 4B.

This same general procedure of measuring visual thresholds may be employed to determine the effects of various other drugs or substances on physiological altitude. Substances which either intensify or ameliorate the effects of high altitude may be studied quantitatively in this manner. This method is limited, however, to the study of drugs which are effective in a relatively short time, up to one or two hours. Since the day-to-day variation of the threshold may be large as compared with the changes during a given experiment, this method does not lend itself so readily to the study of substances which act only after a period of some days.

B. *The mechanism of the action of glucose.* At least two possible mechanisms have been suggested as explaining the "anti-anoxic" effect of glucose: *a*, an indirect action on the brain through an increase in the production of carbon dioxide, and *b*, a direct action of glucose on the rate of oxidation in the central nervous system.

The value of an increased carbon-dioxide tension in counteracting the effects of anoxia under some conditions has been demonstrated (Gellhorn, 1936 and 1937; Gibbs et al.; Ruhl and Spiess). Some authors, such as Ruhl, have suggested that the ingestion of carbohydrates may act like "indirect carbon-dioxide therapy," because of the resultant rise in the R.Q. An increase in the rate of carbon-dioxide production after the ingestion of various sugars has been demonstrated repeatedly. It has generally been observed that the increase in the R.Q. lags behind the rise in blood sugar and persists for some time after the blood sugar level decreases. For example, Cathcart and Markowitz found that although the maximum rise in blood sugar occurred one-half hour after the ingestion of glucose, the R.Q. remained practically unchanged or even fell slightly at the end of the first half-hour, and showed a maximal rise at the end of an hour. If the improvement in visual sensitivity which was demonstrated in the present study were related to the production of carbon dioxide, one might therefore expect that it would not begin until some time after the blood glucose level had risen and would persist considerably longer than the elevation in blood sugar. On the

contrary, the present experiments indicate that the "anti-anoxic" effect of glucose appears coincidentally with the rise in blood sugar, and begins to decline when the blood sugar level drops. It therefore seems reasonable to believe that at least the major part of this effect is related not to the rate of production of carbon dioxide but to the blood sugar level itself.

Glucose plays a unique rôle in the metabolism of the central nervous system. The brain is able to burn only glucose and certain closely related substances. It is believed that when the supply either of glucose or of oxygen is diminished, the oxidative processes of the brain are slowed. There is evidence that a decline in the oxygen utilization of the brain actually occurs during hypoglycemia (Loman and Myerson; Himwich et al.; Loman). Numerous similarities between anoxia and hypoglycemia have been pointed out. Visual thresholds are affected similarly by hypoglycemia and by anoxia. The decline in visual sensitivity due to either of these conditions may furthermore be reversed by the administration of oxygen (McFarland and Forbes; McFarland, Halperin and Niven, to be published). The present studies indicate, moreover, that an excess of glucose may partially compensate for a deficiency of oxygen. These facts suggest that glucose may counteract the visual effects of anoxia directly, by stimulating the oxidative processes in the nervous tissues. No direct measurements of the effect of glucose on the oxygen consumption of the intact brain during anoxia have been reported. Such measurements would be of great value in clarifying the mechanisms involved in the effects demonstrated here.

SUMMARY

1. The measurement of differential intensity thresholds for vision at low brightness levels provides a sensitive and objective index of the impairment caused by anoxia. This test was applied to the study of the modification of this impairment by glucose. Three fasting subjects were used at simulated altitudes of 12,700 to 17,200 feet.

2. The ingestion of 50 grams of glucose during exposure to low oxygen tensions (simulated high altitude) resulted in a considerable decrease in the impairment due to anoxia. At a simulated altitude of 13,800 feet the administration of glucose diminished the visual impairment of one subject to an amount corresponding to an altitude of only 8,000 feet. The "physiological altitude" was thus 42 per cent lower than the actual altitude. In the other two subjects the lowering of physiological altitude was 25 per cent and 48 per cent, respectively.

3. The ingestion of glucose one-half hour before exposure to low oxygen tensions likewise prevented a large part of the impairment expected from anoxia.

4. Control experiments, in which a saccharin solution was given at simulated high altitudes instead of glucose, showed no effects on the impairment caused by anoxia.

5. The administration of glucose to fasting subjects at a normal atmospheric oxygen tension resulted in no improvement of visual sensitivity. An improvement occurred only if visual sensitivity had first been impaired by anoxia.

6. The amount of improvement of visual sensitivity during anoxia after

glucose administration, in relation to time, was approximately parallel to the blood sugar curve during its rise and subsequent fall.

7. The increase in carbon-dioxide production after glucose ingestion lags behind the rise in blood sugar and declines considerably later than the fall in blood sugar. The "anti-anoxic" effect of glucose therefore seems to depend directly on the blood sugar level rather than on the secondary increase in carbon-dioxide production.

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FURTHER AFFERENT CONNECTIONS TO THE ACOUSTIC CORTEX OF THE DOG[1]

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The purpose of this study is to explore the cerebral cortex for the presence of areas for the reception of impulses to sound, other than those reported previously (16). This was suggested by some unpublished findings by Allen in which the auditory cortex of the dorsal and ventral areas was extirpated or coagulated thermally and the dog was able ultimately to re-acquire correct conditioned differential responses with the foreleg to various auditory stimuli. Another area receptive to impulses from the cochlea is described below.

METHODS. Twenty-five dogs were used in these experiments. Dial-Ciba[2] and pentobarbital sodium were employed as the anesthetic agents on alternate experiments. Oscillograms of the cortical and microphonic responses were recorded simultaneously on two cathode ray tubes supplied by two push-pull voltage amplifiers. Stimuli were synchronized with the sweep circuits of these tubes. In the previous investigation (16) the technique was described for stimulating the cochlea with a wavefront of a simple tone, which would produce a response (positive voltage deflection) at an electrode situated within a localized region of the cortex. This method was used again with the following alterations. A velocity microphone replaced the carbon type used earlier. The loudspeaker for tonal stimulation was placed about 30 cm. from the animal's ear. Each point was examined for responses to tones of 100 to 16000 dv.

For electrical stimulation, shocks (condenser discharges) were led through copper wires to the edge of the bony spiral. The thresholds for responses in the dorsal area was observed to assure proper situation of the wires. The modiolus was simply severed by introducing a dental chisel between the basal turn and bony orbit of the cochlea.

The entire dorsal and ventral areas were explored in seven of the experiments of this series with particular attention to the anterior portions located on the anterior ectosylvian gyrus. The cortex here was explored in 2 mm. co-ordinate divisions, as well as the intervening area between the ventral and third area, because of the possibility that this third area might be a part of the ventral and dorsal areas. The third area was examined in 1 mm. co-ordinate divisions to determine if localization could be demonstrated by this means. In the other animals of the series, the intervening cortex was examined in 2 mm. divisions to and including the anterior portion of the ventral area.

Intra-cranial section of the eighth nerve was performed either through an opening in the occipital bone following exposure of the nerve by elevation of the

[1] Aided by a grant from the Committee on Scientific Research of the American Medical Association.

[2] The Dial-Ciba was generously given to the author by the Ciba Company.

cerebellar hemisphere or through the cerebral vault after removal of the cerebral hemisphere and the tentorium cerebelli.

The dorsal and ventral areas were either removed to a depth of 1 cm. with a spatula or coagulated with a hot knife.

It has been established in human beings that, when one tone is impressed on the ear in the presence of a second tone, the second tone has a tendency to obliterate the sensation of the first (11). This fact offered a method for studying the acoustic connections to the cerebral cortex. For the generation of a second continuous tone, another loud-speaker located about 75 cm. from the animal's ear was supplied by an audio amplifier and a resistance-capacity tuning oscillator. The cortical response to the tonal stimuli was observed oscillographically in the presence of the continuous tone at different frequencies. The intensity of the continuous tone was increased until the response to the tonal stimulus was abolished, or if no effect was noted, until the maximum output of the amplifier was attained.

To examine the connections in exclusion of the ear mechanism two single shocks of equal threshold strength were applied in succession through one pair of electrodes upon the bony spiral, and increasing the time interval between the shocks permitted the study of the effect of the first stimulus on the second. The same procedure was followed by applying the first shock to one turn and the second to another turn of the spiral.

Results. The position of this third responsive area (figs. 1 and 2) to tonal and electrical stimulation of the cochlea occupies about 10 to 20 sq. mm. of the cortex at the junction of the anterior ectosylvian gyrus with the coronal gyrus to form the anterior composite gyrus. In several experiments, responses were found extending 1 to 2 mm. upon the margin of the adjacent coronal gyrus. It was isolated from the ventral area by a 6 to 8 mm. unresponsive region in all the experiments of the series, in spite of careful examination of this region of the brain. The isolation of third area from dorsal and ventral areas was especially clear in animals where the anterior suprasylvian sulcus did not reach the third area (fig. 2).

Responses to shocks or tones were obtained from one cerebral hemisphere by stimulation of either ear but the threshold was greater for the same side. Also, the threshold for this area was higher than for the dorsal and ventral areas. The threshold was markedly lowered by lightening the anesthesia to the point where lid reflexes were evident. The responses to either tonal or electrical stimulation were characteristically positive voltage deflections appearing 7 to 10 msec. after the moment the stimulus was applied (figs. 3–5). The time of appearance in general coincided with responses in the dorsal area (fig. 4). The amplitude of the deflection reached a maximum of 100 to 200 micro-volts in the central portion of the area, diminishing toward the periphery.

To determine the origin of these responses, experiments brought out the following facts: 1. Destruction of the dorsal and ventral areas did not affect the responses. 2. Complete intra-cranial division of the eighth nerve abolished the responses in all three areas. 3. In several experiments after outlining the cortical

area to shocks applied to the apical, middle, and basal turns, the wires were placed 4 mm. lateral, medial, and posterior to the basal turn. No responses were obtained from this region of the cortex. With the wires 2 mm. laterally upon the facial nerve, twitches of the face muscles became apparent, coincident with a sharp oscillographic deflection bearing a latency of 2 to 3 msec. Adequate evi-

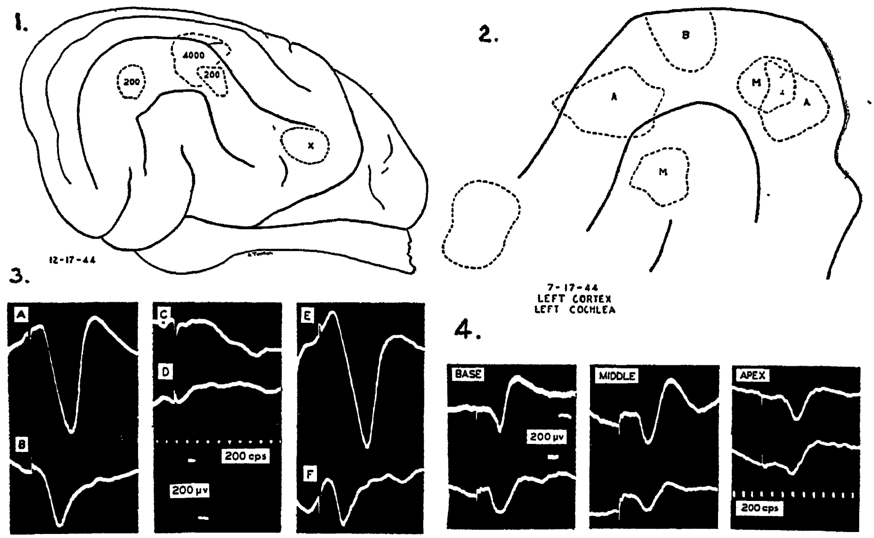

Fig. 1. Right cortex; left ear. *X* indicates third area from which responses were obtained to tonal stimuli. Above are outlined the responsive foci for tones of 200 dv. in the dorsal and ventral areas, and 4000 dv. in the dorsal area. No responses to any frequencies in region between the third and ventral areas.

Fig. 2. At lower left, third area outlined to electrical stimulation of the apex, middle, and base of the cochlea. Above, the responsive foci for the apical, *A*, middle, *M*, and basal, *B*, turns are outlined in the dorsal area, as well as the foci for the apical and middle turns in the ventral area. The representation of the basal turn in the ventral area was not located in this animal, although the middle sylvian and posterior ectosylvian gyri were examined in detail. The region between the third and ventral areas was entirely unresponsive to stimulation of the apical, middle and basal turns.

Fig. 3. 11/29/44. *A*, *C*, *E:* from point in dorsal area. *B*, *D*, *F:* from point in third area. Shocks delivered to bony lamina of middle turn, before (*A*, *B*) and after (*C*, *D*) section of modiolus. *E*, *F:* shocks applied to central stump of 8th nerve. Downward deflection in this and succeeding figures: surface positive.

Fig. 4. 4/2/44. Comparative examples of responses. Upper records: from the dorsal area. Lower records: from a single point in the third area. Stimulation by means of electric shocks. Note latencies of the responses.

dence showed that this was a direct muscle potential. 4. Responses in the area from direct electrical stimulation of the middle or apical portions of the spiral were abolished by severing the modiolus. Replacing the electrodes upon the central stump of the eighth nerve caused the responses to reappear (fig. 3).

A spatial pattern of localization was not clearly evident although there was a tendency for larger potentials to be seen from the anterior and superior portion to

low frequencies and from the posterior and inferior to high frequencies. In one instance, electrical stimulation demonstrated three points 2 mm. apart, representing the apex, middle and base, in conformity to the arrangement suggested by the results to the tones above.

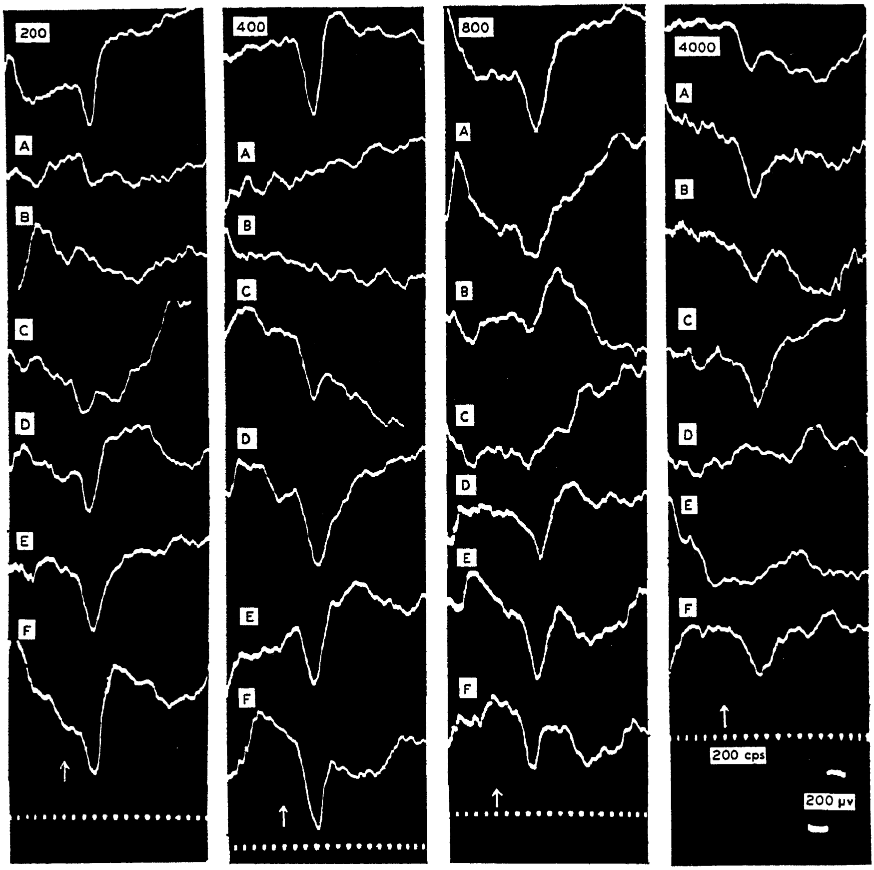

Fig. 5. Oscillograms from single point marked X in third area of figure 1. Each column represents responses to tonal stimuli of frequencies indicated at the top. First record in each column taken without the presence of the continuous tone. Succeeding records with the presence of a continuous tone: A, 200; B, 400; C, 800; D, 2000; E, 4000; F, 8000 dv. Arrows indicate time of arrival of the wavefront at the animal's ear. Note absence or reduction of responses when stimulus and continuous tones possess about the same frequency.

A central point within the area was responsive usually to tones possessing a range of frequencies from 200 to 8000 dv., less often for 100 and 16000 dv. The responses to the tonal wavefronts were readily abolished by the presence of a continuous tone of about equal intensity with a frequency differing by an octave or so from the stimulus tone. Examples of this effect are shown in figure 5; 2000 and 8000 were omitted from the figure, but the results for these were the same. The response to a pulse of 2000 dv. was abolished by the tones of 800, 2000, and

4000 dv., while the response to 8000 dv. was prevented by 4000 and 8000 dv. The tones greater than an octave from the stimulus tone at their full intensity had no apparent effect.

Preliminary experiments on the dorsal and ventral areas have shown that a similar obliteration of the threshold responses to the tones occurs in the presence of the continuous tones and at intensities comparable to those in this new area.

Application of two successive electrical shocks to one turn of the bony spiral resulted in the obliteration of the responses to the second shock in both dorsal and third areas for a period of 60 to 100 msec. after the first. When the first shock was delivered to the middle turn, and the following shock to the basal turn, no change in size of the response was noted in the basal region of the dorsal area. Likewise, in the third area, although responses from both parts of the cochlea were obtained from the same cortical point, no effect on the second response was found.

DISCUSSION. The investigations on the effects of cortical lesions on hearing in dogs by Munk (12), Alt and Biedl (2), Kalischer (5), Rothmann (14), Swift (15) and Pavlov (13) do not include this area. One of Luciani's (8) animals (dog S) was deaf as result of multiple lesions involving the posterior halves of the brain, including this region on one side. However, his hearing tests were probably inadequate for the study of cortical function.

The proximity of the area to the tactile system has received particular attention because of the possibility of exciting tactile endings either directly or reflexly. Marshall, Woolsey, and Bard (10) have obtained responses in this region in the cat to bilateral tactile stimulation of the forepaws. General cutaneous representation of both fore and hind paw was found by Adrian (1) in the cat and dog but only on the contralateral side. The latter investigator found the sensory component of the fifth nerve represented on the coronal gyrus.

In the present experiments, the effects of cutting the modiolus and eighth nerve place the site of stimulation in the cochlea. However, reflex arcs, particularly arising from the tensor tympani and stapedius muscles were considered. Optical measurements by Kato (6) and Lorente de No (7) in cats and rabbits have shown a minimum response time of 10 to 12 msec. and a maximum of 40 msec. for such reflexes. In view of the latency of 7 to 10 msec. for the cortical responses to sound and the electric shocks, it seems unlikely that reflexes of that nature are of concern. Furthermore, the specific obliterating effect of the continuous tone and the behavior of the responses to the successive shocks are analogous to the conditions observed for the dorsal area.

Localization is not spatially evident in all experiments, but the obliteration of the responses to tonal stimuli by the continuous tone of about the same frequency leads to the conclusion that separate fibers from different parts of the cochlea enter this region of the brain. Combinational tones and harmonics may be present but do not invalidate this conclusion. The absence of inhibition by the first electric shock on the second when delivered to separate turns of the cochlea also lends support.

The phenomenon of obliteration of the sensation of a tone by another is termed masking and has been investigated fully by Wegel and Lane (17) in human beings.

The preliminary data offered here support their findings from an anatomic basis, although masking in these experiments is limited to only two octaves. The data also support the conclusion that the masking effect is a phenomenon associated with the physical mechanism of the cochlea.

This part of the brain lies in a region from which Bechterew and Mislawski (3) and others by faradic stimulation obtained salivary secretion mediated by the chorda tympani. By stimulation of two distinct points, Mann (9) obtained front and hind leg movements, and by stimulation of the two points, a walking or swimming motion. Garol (4) has confirmed the excitability of this part of the cortex.

CONCLUSIONS

An area, bilaterally receptive to impulses from the cochlea, has been described occupying the ventral end of the anterior ectosylvian gyrus in the cerebral cortex of the dog.

The use of masking and electrical stimulation of the cochlea furnished the evidence for the existence of separate fiber connections from local parts of the cochlea to this area.

Acknowledgment. The author is indebted to Dr. Robert S. Dow and Mr. Fred Claussen for their encouraging assistance with the problem.

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THE CHANGES IN RENAL CLEARANCE FOLLOWING COMPLETE ISCHEMIA OF THE KIDNEY[1]

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Renal clearances in dogs are markedly reduced in experimentally induced shock (limb compression), in hypotension resulting from hemorrhage (1, 2) and in human traumatic and hemorrhagic shock (3). From the low clearance values of inulin and mannitol, it is concluded that glomerular filtration is reduced, and likewise, since the clearance values of diodrast and p-amino hippurate are low, that the effective renal plasma flow is reduced. These results have been interpreted as indicating that vasoconstriction and reduced blood flow exist in the kidney under these conditions.

One difficulty in making exact renal hemodynamic interpretations from the clearances during hypotension has been pointed out by Corcoran and Page (2) who found that at a mean blood pressure level of about 60 mm. Hg the effective intraglomerular filtration pressure drops to 0; at this critical level glomerular filtration is arrested by the osmotic pressure of the plasma proteins, and excretory function ceases, so that observations of clearance and renal extraction no longer measure renal blood flow. Furthermore, since anoxic conditions are apt to prevail in the kidney during shock conditions, the likelihood exists that normal glomerular and tubular mechanisms may become impaired, thus further invalidating the clearance technique for quantitative estimates of renal blood flow. The report that the plasma extraction ratio of diodrast decreases during the circulatory depression following limb tourniquet release and remains low after the re-infusion of blood following hypotension appears to support this view (1, 2). Corcoran, Taylor and Page have accordingly qualified their interpretation of diodrast clearance as a measure of renal plasma flow under the above conditions. Lauson and his co-workers (3) have likewise fully considered the possible limitations of the clearance method in shock.

In order to further explore the value and limitations of renal clearance as an index of renal blood flow under anoxic conditions, the effect of temporary ischemia on the clearance of p-amino hippuric acid and creatinine by one kidney of the dog was investigated, while the opposite kidney served as a control.

Experimental procedure. Usually female dogs ranging in weight from 8.5 to 18.5 kgm., anesthetized intravenously with 30–35 mgm. per kgm. of body weight of pentobarbital sodium, were used in the present experiments. The left kidney was approached by a ventral, occasionally by a dorsal incision. The left ureter was cannulated, and the left renal vein and artery were gently dissected sufficiently away from surrounding tissue to allow placement of loose ligatures. The assumption is made that the nervous supply to the kidney has

[1] Supported by a grant from the Commonwealth Fund.

not been seriously interfered with. Femoral arteries were cannulated for continuous registration of mean blood pressure and for withdrawal of arterial blood for analysis of the infused substances; one femoral vein was cannulated for infusion. An indwelling catheter in the urinary bladder permitted collection of urine from the right kidney. The bladder was rinsed with saline to insure accurate emptying at the termination of each urine collection period. Since it was found difficult to empty the bladder in male dogs, they were not catheterized for right kidney urine flow. Following the operation and preceding the clearance determinations, approximately 200–400 cc. of 0.9 per cent saline were infused to aid in maintaining an adequate urine flow.

Following a priming dose of 0.25–0.5 gram of creatinine and 100–200 mgm. of p-aminohippuric acid (PAH), constant plasma levels were maintained by continuous intravenous infusion of 0.9 per cent saline containing an average concentration of 0.77 per cent creatinine and 0.190 per cent PAH at the rate of 1.3 cc. per minute. In some experiments, an osmotic diuretic, mannitol, was added to the infusion fluid in concentration suitable to deliver 13.5 mgm. per kgm. per minute, following a 5 gram priming dose.

About an hour after the surgical procedures, two control urines were collected for periods of approximately 15 minutes each. These were followed immediately by application of a clamp to the left renal artery. The periods of occlusion lasted 3–5, 10 and 20 minutes respectively in different experiments. After release of the clamp at least 20 minutes were allowed for flushing stagnant urine out of the tubules and collecting system before urine collection was resumed. In a few experiments, following a five minute clamping procedure, sufficient time was allowed for recovery and then the arterial clamp was replaced for longer intervals (10 or 20 min.).

To determine clearances, creatinine and PAH concentrations obtained from frequent arterial samples, usually one per urine collection period, were interpolated to the midpoint of each urine collection period corrected for emptying time. In order to determine the extraction ratio of PAH, renal vein blood samples were drawn by direct puncture simultaneously with the arterial samples. Plasma proteins were precipitated by the $CdSO_4$ method (4). Creatinine was analyzed in a photo-electric colorimeter by the alkaline-picrate method of Folin and Wu (5), and the p-aminohippuric acid was analyzed by Smith's modification (6) of the method of Bratton and Marshall (7).

Results. *A. Control clearance data and observations on the mean blood pressure.* Table 1 shows first a comparison of clearances in trained, unanesthetized dogs with those obtained in operated, anesthetized animals. While control clearance values are somewhat higher in the latter, the differences are not sufficiently large to invalidate the use of anesthetized and operated dogs in these studies.

A comparison of the control clearances of the left kidney with those of the right kidney shows no significant differences. Since the right kidney was the unoperated control, this indicated that the surgical procedure in the vicinity of the left kidney had no detectable influence on the left renal function.

The control right renal clearances were continued in 13 experiments (74 observations) for an average time interval of 3.6 hours from the beginning of the experiment (range: 120 to 320 min.). During these observations the left renal artery was clamped and the subsequent changes in left kidney function were produced. When these right kidney observations are compared with the control data, it is seen that neither creatinine nor PAH clearances show a significant change. The conclusion that the changes in left renal function to be described are largely restricted to that kidney is further supported by the lack of significant alteration in the systemic mean blood pressure. The mean of all control measurements was 135 mm. Hg (range 104 mm. to 155 mm.); 84 per cent of 496 observations remained within ±15 per cent of the control means and the limits of variation were ±30 per cent.

B. Effect of clamping of the left renal artery on the left renal clearances. 1. *Three to five minutes of ischemia.* Nine experiments were performed in this series in four of which mannitol diuretic was employed. Since the results did not differ, they are grouped together in the figures and tables. Because there was considerable variation from dog to dog in average control clearance values, the experimental data are plotted in figure 1 as a ratio of clearances following ischemia to the control clearances. The upper solid line of figure 1 shows that a period of complete ischemia lasting up to five minutes produced only a transient decrease in renal clearances, returning approximately to the control mean value within 120 minutes after the release of the clamp (ratio to the control mean of 0.85 for p-aminohippuric acid clearances and 1.02 for creatinine clearances at 120 min.).

2. *Ten minutes of ischemia.* The lower solid line of figure 1 shows that when the renal artery was clamped for 10 minutes in three experiments there was an initial profound decrease in the clearances, followed by a gradual partial recovery during the period of observation. At 120 minutes the creatinine clearance had returned to only 30 per cent of the control mean, and the PAH clearance to 44 per cent of the control.

3. *Twenty minutes of ischemia.* The solid circles at the bottom of figure 1 show that with no osmotic diuretic, a period of ischemia of 20 minutes (3 expts.) virtually abolished the renal clearance of creatinine and PAH during observations extending to 136 minutes after the release of the renal arterial clamp. Profound changes in urine flow from extreme oliguria to anuria were associated with the reduced clearances.

When the osmotic diuretic, mannitol, was employed the results were significantly different (table 2). Anuria was not observed, and although the results were variable, the clearances were consistently better following twenty minutes of ischemia than without mannitol. Thus, the average experimental ratios to the mean control values during periods of observation lasting as long as 175 minutes after the release of the clamp were: PAH, 0.42 (range, 0.117 to 0.87) and for creatinine, 0.345 (range, 0.08 to 0.78). As can be seen in the table, the improvement in clearances was directly related to the increase in urine volume resulting from the osmotic diuretic action.

C. The changes in urine flow resulting from ischemia. Figure 2 demonstrates that there is a definite relationship between the changes in renal clearance and

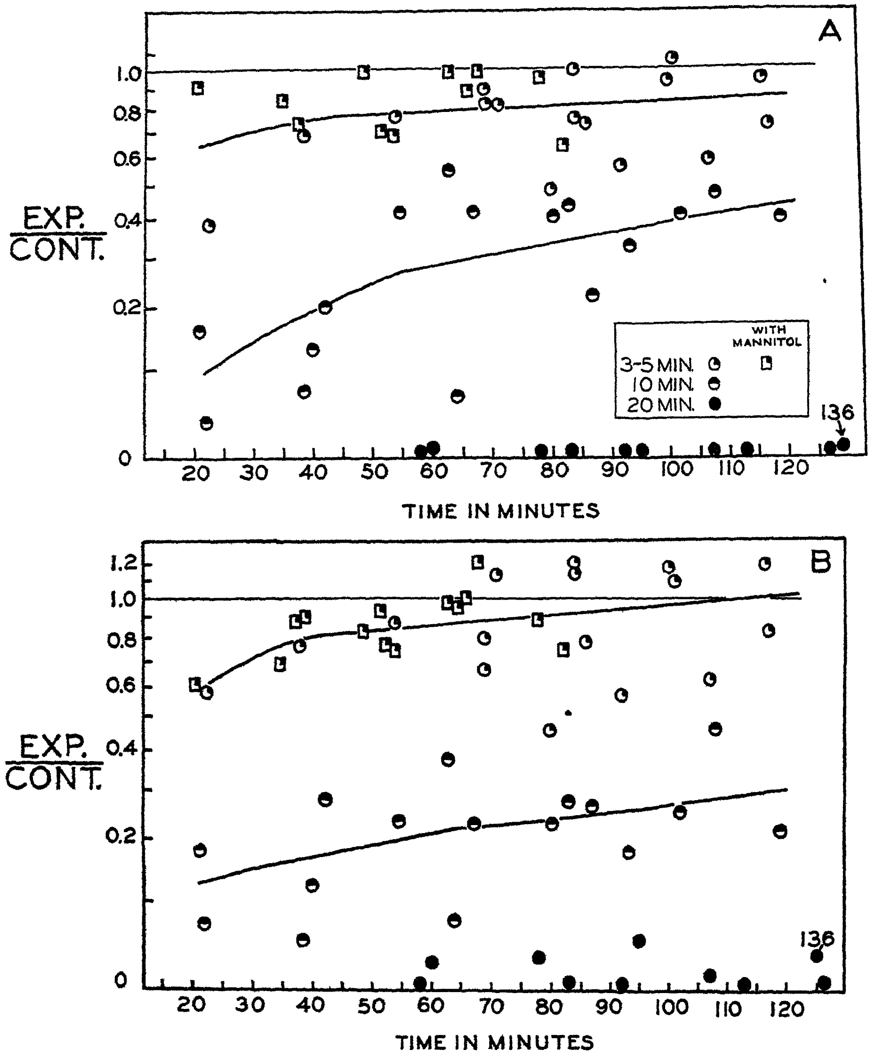

Fig. 1. A. The changes in renal plasma clearance of p-aminohippuric acid following complete renal ischemia produced by clamping the renal artery. Each symbol is the midpoint of one experimental urine collection period expressed as a ratio to the mean of two control periods. The solid lines represent the best fits of the arithmetic means of the results of 3–5 (upper line) and 10 minute periods (lower line) of ischemia respectively. Several periods of complete anuria resulting from a 20 minute period of ischemia (solid circles at bottom) have been reported as "O" clearance. Time is in minutes after release of clamp.

B. The changes in renal plasma clearance of creatinine following complete renal ischemia.

the rate of urine flow. Following a 3–5 minute period of ischemia, the relation of clearance to urine volume is similar to the control data in that they are rela-

tively independent of each other. However, 10 minutes of ischemia markedly reduces the clearances of both creatinine and PAH and the urine volume is cor-

TABLE 1

Comparison of control clearance data

	NO. OF OBS.	*p*-AMINO HIPPURIC ACID CLEARANCE cc./min. per kidney/sq.m. S.A.*			CREATININE CLEARANCE cc./min. per kidney/sq.m. S.A.		
		Mean	S.D.§	S.E.	Mean	S.D.	S.E.
Unanesthetized control†	63	124	29.6	3.74	41.4	7.45	0.94
Left control kidney	34	140	44.0	7.65	47.0	17.0	2.92
Right control kidney	30	142	48.0	8.93	51.7	17.0	3.10
Right kidney following left renal artery clamp‡	74	128	43.0	5.00	52.0	19.7	2.29

* Surface area was determined according to the Meeh-Rubner equation: surface area in square meters $= \dfrac{11.2 \times W^{2/3}}{100}$, W = weight in kgm.

† All data were obtained in four trained female dogs.

‡ These clearances followed the period of clamping of the left renal artery simultaneous with experimental changes in left renal clearance.

§ S.D. = standard deviation from the mean; S.E. = standard error.

TABLE 2

*Changes in left renal clearance with mannitol diuretic following a 20 minute period of clamping of the left renal artery**

EXP. NO.	TIME IN MINUTES AFTER RELEASE OF ARTERIAL CLAMP	NO. OF PERIODS	URINE FLOW	p-AMINO HIPPURIC ACID CLEARANCE cc./min. per kidney/sq.m. S.A.	p-AMINO HIPPURIC ACID CLEARANCE Exp./Cont.	CREATININE CLEARANCE cc./min. per kidney/sq.m. S.A.	CREATININE CLEARANCE Exp./Cont.	Creatinine U/P
			cc./min.					
1	Control:	2	1.680	158.0		39.0		16.1
	48-88	3	0.340	18.5	0.117	3.2	0.082	6.27
2	Control:	2	1.930	177.0		60.0		18.42
	18-71	4	0.81	50.0	0.283	9.3	0.157	6.80
3	Control:	2	1.47	117.0		41.5		16.5
	49-175	3	0.82	48.7	0.416	15.0	0.362	10.2
4	Control:	2	1.031	103.0		54.7		38.4
	32-75	3	1.165	89.7	0.870	42.7	0.780	28.7

* All figures in the table represent the average values of the control periods and the periods following ischemia in each experiment.

respondingly reduced. Twenty minutes of ischemia produces in some cases complete anuria, thus abolishing entirely the plasma clearance of creatinine and PAH.

D. Changes in concentrating ability of the renal tubules. In figure 3 are compared the concentration ratios (U/P) for creatinine during the control and following ischemia. Arbitrarily selecting control urine flows of less than 0.15 cc. per minute, the U/P ratio is high, averaging 336 for 8 observations (range, 232 to 477). But following the 10 minute period of ischemia with the same range of urine flows, the average U/P ratio of 11 observations is only 126 (range, 58 to 250). Following 20 minutes of ischemia, the changes are even greater, but due to the extreme oliguria too much significance cannot be attached to the latter data.

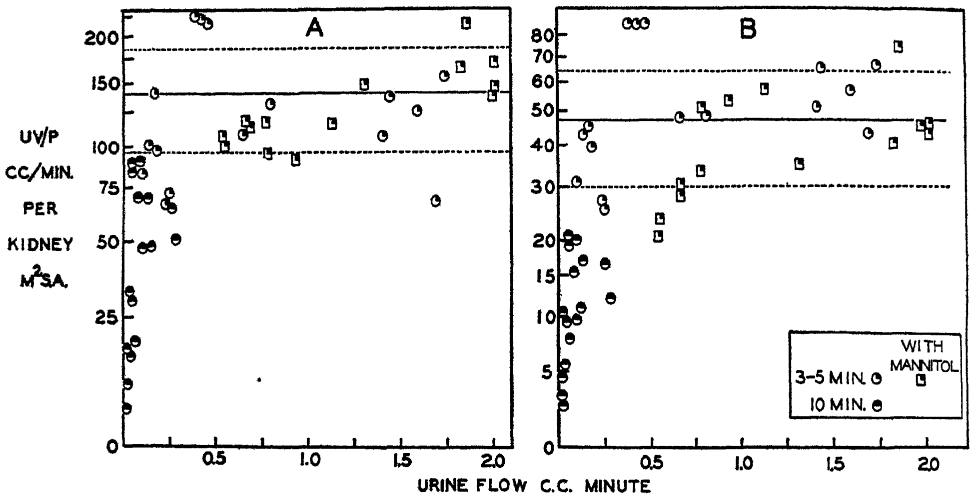

Fig. 2. A. Showing the relationship of the renal plasma clearance of p-aminohippuric acid to the rate of urine flow. The clearances are expressed as cc. per minute per kidney per square meter of surface area. The solid line represents the mean of the left control kidney clearances (140), and the dotted lines represent the standard deviation (±44). To avoid crowding, the results of 20 minutes of ischemia have been omitted from the figure; the calculated means of the latter are: p-aminohippuric acid clearance, 0.435 cc./min. per kidney/sq.m. S.A.; urine flow, 0.013 cc./min.

B. Showing the relationship of the renal plasma clearance of creatinine to the rate of urine flow. The mean of the control observations is 47, the standard deviation is ±17. The mean clearance following 20 minutes of ischemia was 1.0 cc./min. per kidney/sq.m. S.A.

The concentration ratio of PAH is similarly influenced by ischemia, but because of the participation of tubular excretion in its renal clearance, analysis of the cause of this decrease is complex. This change may be the direct result of decreased efficiency of the tubules to excrete the PAH as evidenced by the decrease in extraction ratio in proportion to the length of ischemia (table 3). The fact that the extraction ratio and the clearance of PAH are improved by the use of the osmotic diuretic, because of the resulting increase in urine volume, suggests that PAH may be excreted in a normal manner but indiscriminately stored or reabsorbed elsewhere in the renal tubules.

E. Histological changes resulting from renal ischemia. Two kidneys which had

undergone 20 minutes of ischemia were examined histologically;[2] in one of these experiments mannitol diuretic had been given (expt. 2 in table 2). Both kidneys exhibited a nephrosis characterized by cloudy swelling and hydropic degeneration of the tubular epithelium. These changes were most marked in the periphery of the cortical area. Some debris appeared in the tubular lumens. The changes appeared less marked in the kidney of the animal that had received the mannitol diuretic. There was no histological evidence of edema.

Discussion. It is customary to interpret reduction in the clearance of inulin (or creatinine in the dog) and diodrast or PAH as being indicative of vasocon-

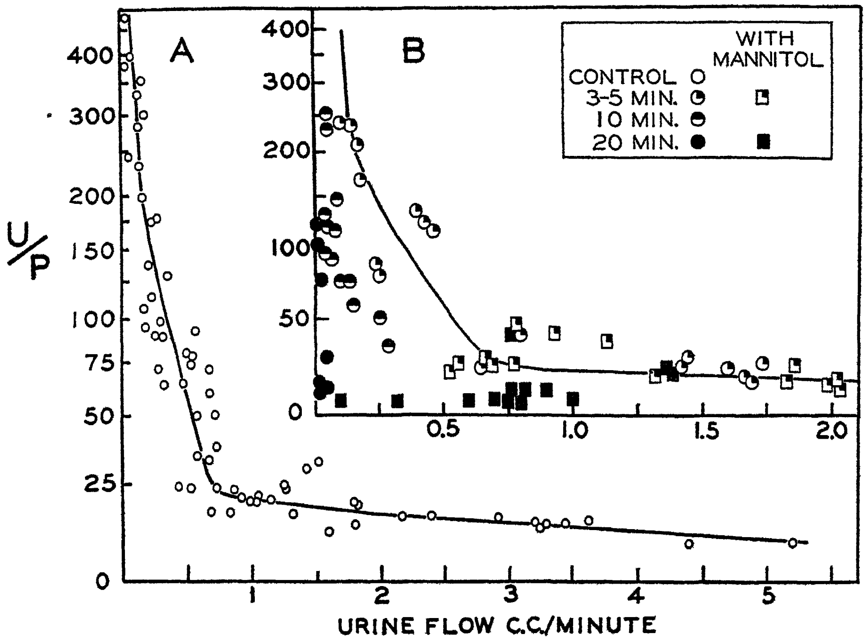

Fig. 3. Showing changes in U/P of creatinine following various degrees of complete renal ischemia. The control data in A are derived from both right and left control kidney observations and include data in which mannitol diuretic was employed. The curve represents the best fit of the arithmetic means of the control data. In B the changes in U/P ratio resulting from ischemia are shown in relation to the mean control curve. Several periods of extreme oliguria and anuria have been omitted from this figure.

striction in the kidney (8). Following the longer periods of anoxia produced by clamping of the renal artery the marked decreases in creatinine and PAH clearance may thus be interpreted as being the result of prolonged vasoconstriction of the renal arterioles. Since similar degrees of reduction occur in both creatinine and PAH clearance, this would imply an afferent arteriolar constriction (or simultaneous afferent and efferent constriction). Marked afferent arteriolar constriction, resulting in reduced glomerular filtration pressure, could account for the related reduction in urine flow.

[2] We are indebted to Dr. Harry Goldblatt for examination of the histological preparations.

However, it is believed that anoxia creates conditions in the renal tubules which exaggerate the apparent vasoconstriction as judged by the renal clearances. The extraction ratio of PAH is reduced in proportion to the length of ischemia, indicating impairment of the excretory ability of the tubular cells. Such impairment would exaggerate the decrease in apparent renal plasma flow. Furthermore, the decrease in the U/P ratio of creatinine occurring with reduced urine flows suggests the probability that creatinine is being reabsorbed by the tubular cells, rendering invalid its clearance as a measure of glomerular filtration rate. It seems a fair assumption that the related oliguria may be in part the result of damage to the tubular cells similar to the hyposthenuria and oliguria following uranium poisoning in the dog (9); here the damaged cells act like a "dead" membrane, and the glomerular filtrate may be completely reabsorbed, the absorbing force being the osmotic pressure of the plasma colloids of the peritubular capillaries. It is of interest to note that following the 20 minute period of renal ischemia the tubular histological changes resemble a mild degree of uranium poisoning. The improved urine flow following the use of the osmotic diuretic mannitol, by competing for the glomerular filtrate with the osmotic pressure of the plasma colloids, would appear to favor the above interpretation. It appears likely that oliguria and anuria following the longer periods of ischemia are the result of both arteriolar vasoconstriction and the tubular changes described.

TABLE 3

Summary of changes in p-amino hippuric acid extraction ratio

	NO. OF OBS.	EXTRACTION RATIO*		PLASMA CONC. OF p-AMINO-HIPPURIC ACID	
		Mean	Range	Mean	Range
				mgm./%	*mgm./%*
Control†	15	0.645	0.515–0.758	1.64	0.780–2.96
3–5 min. clamp†	15	0.590	0.308–0.783	1.35	0.725–2.06
10 min. clamp	9	0.380	0.235–0.534	3.00	2.440–3.63
20 min. clamp					
no mannitol	8	0.118	0.00 –0.413	6.53	4.60 –10.0‡
with mannitol	7	0.425	0.22 –0.620	1.83	1.50 –2.37

* Extraction ratio $= \frac{A - V}{A}$ (A = arterial plasma conc. in mgm. per cent; V = renal venous plasma conc. in mgm. per cent).

† These data include experiments with mannitol diuretic.

‡ Constant rates of infusion of PAH were maintained throughout these experiments. The plasma concentration rose to these levels because of reduction of renal clearance.

Because reduced blood flow exists in hemorrhagic shock, the probable rôle of anoxia in bringing about renal changes and impairment under these conditions should be evaluated. It is suggested that caution be exercised in the use of renal clearances in evaluation of renal hemodynamics under conditions in which hypotension and anoxia occur.

SUMMARY AND CONCLUSIONS

Experiments performed on anesthetized dogs showed that changes in renal function analogous to those seen in hypotension and shock result from temporary complete renal ischemia produced by clamping the renal artery. These changes occur while the mean blood pressure is maintained at normal levels. It was found that the clearance of creatinine, taken as a measure of glomerular filtration in the normal dog, and the clearance of p-aminohippurate, a measure of effective renal plasma flow, may be markedly reduced following brief periods of complete ischemia, the length and degree of the reduction being in direct proportion to the length of the period of clamping.

The results indicate that prolonged vasoconstriction of the renal arterioles follows the period of ischemia, thus playing a rôle in the decreased renal clearances. In addition, anoxia resulting from the ischemia produces additional changes in the clearances referable to damage of the renal tubules.

Decreased urine flow after ischemia is associated with the reduced clearances. This is thought to be due to arteriolar vasoconstriction which reduces glomerular filtration, and to renal tubular damage, which modifies the normal tubular water reabsorptive mechanism.

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THE ELECTROCARDIOGRAM IN CHRONIC THIAMINE DEFICIENCY IN RATS

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Cardiac abnormalities as determined by clinical and electrocardiographic observations have been reported in both man and experimental animals as a result of thiamine deficient diets. There has been, however, little agreement as to the nature of the defects in the various species. These reports have been reviewed and discussed in other publications (1, 2, 3, 4, 5).

Recently, Wintrobe et al. have published a study of induced chronic thiamine deficiency in swine (3). Using purified diets and crystalline vitamin supplements, they found pronounced electrocardiographic changes. Bradycardia, prolonged P-R interval, second degree A-V block, P wave changes, inverted T_4, nodal and ventricular premature beats, auriculo-ventricular dissociation, complete heart block and one case of auricular fibrillation were recorded. Areas of necrosis, cellular infiltration and scarring, cardiac hypertrophy and dilatation in the auricles and ventricles of these pigs were reported. These marked histologic changes undoubtedly account, at least in part, for the electrocardiographic changes.

Waisman and McCall (5) have recently studied the electrocardiograms of monkeys which were fed purified diets with crystalline vitamin supplements in addition to liver extract treated with sulfite to destroy thiamine. They noted a marked bradycardia, decreased height of R waves and flat or inverted T waves in nearly all animals. A few showed a wide and notched QRS complex, and a shift in the pacemaker. Most of these changes were reversible under thiamine therapy. Histologic studies of the heart were not reported.

Ashburn and Lowry (4), using purified diets and crystalline vitamin supplements, produced a chronic thiamine deficiency in albino rats. They observed a high incidence of lesions in the auricles and pulmonary veins as well as a few similar lesions in the ventricles. Other investigators have described various lesions in the hearts of experimental animals deficient in thiamine but only the studies of Porto and deSoldati (6) in dogs, Follis et al. (7) in swine, and Lowry and Ashburn (8, 4) in rats have described lesions in the auricular myocardium.

Operating on the assumption that such pronounced auricular pathology should cause more specific defects in heart rhythm and impulse conduction than had been reported in rats, King and Sebrell (9) studied 24 pairs of the albino rats used by Ashburn and Lowry (4) in the second phase of their study. They found four rats with auricular fibrillation and four with A-V nodal rhythms. They also mentioned that other abnormalities such as premature beats, ectopic beats, auricular standstill, shifting pacemaker and increased P-R interval were encountered.

The work reported here was undertaken to reproduce King and Sebrell's observations and to attempt to increase the incidence of auricular fibrillation and A-V nodal rhythms by altering the experimental conditions to produce a more chronic thiamine deficiency. King and Sebrell's findings were corroborated but the incidence of these two abnormal rhythms decreased rather than increased. This report will describe the various electrocardiographic defects mentioned but not described by King and Sebrell, as well as another finding, not previously reported, (bundle branch block) which appeared early in the deficiency before signs of acute thiamine deprivation were evident.

METHODS. The basic diet and vitamin supplements are the same as described in Ashburn and Lowry's experiments (4). The diet was composed of leached and alcohol extracted casein, 18; sucrose, 73; Wesson oil, 3; cod liver oil, 2; Osborne and Mendel salt mixture, 4. Crystalline vitamins were given by supplement dish in the following amounts daily: pyridoxine 20 micrograms, riboflavin 50 micrograms, calcium pantothenate 50 micrograms, nicotinic acid 1 mgm. and choline chloride 20 mgm. The thiamine intake was varied as described later.

Fifty-six albino rats of N.I.H. strain were divided equally into four groups being matched in each according to weight, age, sex and litter.

Each of these four groups received diets and vitamins as indicated above with following additions:

Group I—100 micrograms of thiamine daily.

Group II—100 micrograms of thiamine daily; 10 per cent of the sucrose in the diet was replaced by 10 per cent whole dried liver.

Group III—3 micrograms of thiamine daily.

Group IV—2 micrograms of thiamine daily.

Rats were placed on experiment at weaning (21–25 days). The control animals (groups I and II) were maintained on the regimen mentioned without change. The experimental animals (groups III and IV) were kept on their respective low thiamine intakes for 8 to 10 weeks (av. 9). At the end of this period all thiamine was withdrawn. As the rats developed acute episodes of polyneuropathy (ataxia, spasticity and convulsions), they were treated with 25 to 50 micrograms of thiamine and then allowed to develop another episode. A few animals were allowed to develop a third attack.

Those which died and others which were killed at various stages of the experiment were autopsied and the tissues examined histologically. The few animals which developed lung or other intercurrent infections were excluded from the study.

Electrocardiograms were taken on both control and experimental animals at weekly or biweekly intervals. When the animals showed acute deficiency symptoms, electrocardiograms were taken at frequent intervals just before and after thiamine therapy.

Electrocardiograms were obtained using a string galvanometer with a 3 stage resistance-capacity coupled Sanborn "Cardioscope" amplifier in the circuit. The apparatus was standardized so that 1 mv. produced 2 cm. of string deflection. With this arrangement, the sensitivity was well in excess of the minimum neces-

sary to record the very rapid action of the rat's heart and the deflections had sufficient amplitude to permit detailed study. The camera speed was increased from 25 to 75 mm. per second to spread the various components enough to permit accurate measurement of the P-R and QRS intervals.

The three standard leads were recorded. Contact with the extremities was made by winding fine copper wires around the limb after the fur had been smoothed down with electrode paste. Rats were held in their normal crouched position by hand, short circuiting being prevented by rubber gloves. No anesthetics were used. After a few times rats would become accustomed to this procedure and tracings reasonably free of muscular interference could be obtained readily.

The electrocardiogram of normal rats. The heart rate of the rats in groups 1 and 2 (controls) varied considerably. On the average it was 500 to 525 per minute during the first weeks after weaning and thereafter fell gradually to around 475 per minute (see fig. 1). Rates of 600 per minute were not uncommon and occasionally a rat would show a heart rate as low as 330.

The contour of the tracings was not unlike human electrocardiograms. Lead 1 was very unstable and little significance could be attached to the form, amplitude or direction of its various components, although most often they were upright. Lead 3 was quite stable and lead 2 was very stable. In these leads the P, R and T waves were always upright. S waves were common, Q waves rare.

At rates below 400 per minute a mild sinus arhythmia was common, above this the rhythm was very regular.

The P-R interval varied from 0.035 to 0.05 sec., in different rats, increasing gradually with age. The QRS complex ranged from 0.006 to 0.013 sec. in duration, also increasing gradually with age. The Q T interval had a duration of 0.05 to 0.09 sec. varying inversely with heart rate. An S T segment was not seen except in very slow heart rates. The takeoff of the T wave occurred directly after the R or S waves and is commonly elevated 1 to 4 mm. in leads 2 and 3, depressed 1 to 2 mm. in lead 1. The P wave commonly supervened before the T wave returned to the isoelectric line. Individual normal rats usually showed very similar tracings from day to day and week to week. The changes occurring with age were gradual.

Results. Figure 1 depicts the average weight gain of the various groups in relation to their average heart rates. The control animals receiving liver gained slightly more than those not receiving it, but the heart rates of the two groups were almost identical. No abnormalities were noted in the electrocardiograms of these control animals and the tracings were similar in all respects to those of rats on a good stock diet.

It will be noted (fig. 1) that the heart rates of the rats on low thiamine intake began to fall while the rats were still gaining weight at an almost normal rate. In addition, the rates for the rats receiving only 2 micrograms of thiamine per day (group IV) decreased more quickly and to a greater extent than did those receiving 3 micrograms (group III) although in the early stages the two groups gained weight at almost the same rate.

The quantities of thiamine given to groups III and IV were sufficient to prevent accute deficiency episodes in all but one rat. This animal (20358) developed ataxia, spasticity and convulsions after 60 days on 3 micrograms of thiamine daily. No significant differences were observed in the electrocardiograms of the rats in group III and group IV, consequently they will be discussed together.

During the 8 to 10 week period of low thiamine intake, the electrocardiograms taken were normal, except for the moderate bradycardia, in all but 2 rats. Rats 20351 and 20364 after 33 and 21 days, respectively, on the low thiamine regimen

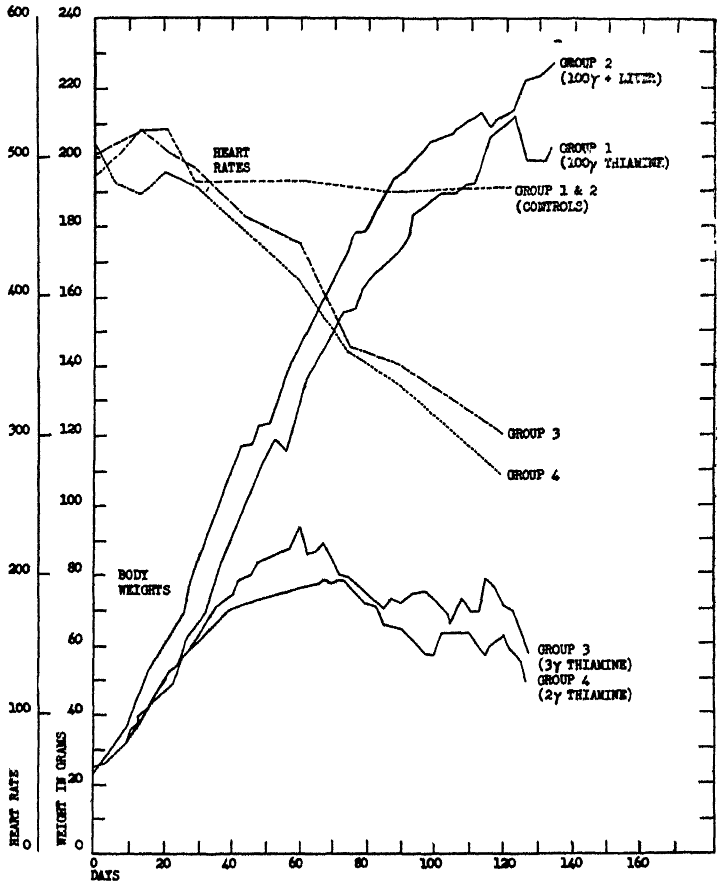

Fig. 1. Average weights and heart rates of the various groups

developed unexpected sharp weight losses accompanied by a reluctance to move. They were not observed to eat or drink. When pushed on their sides or back they regained their feet slowly. They responded slowly to stimuli. There was no detectable paralysis, however, and no spasticity or convulsions. Electrocardiograms showed in both, a bradycardia with a bundle branch block and first degree A-V block (20351 shown in fig. 2). No treatment was administered and in 4 to 8 hours the rats began to resume normal activity. Electrocardiograms showed a return to normal (fig. 2).

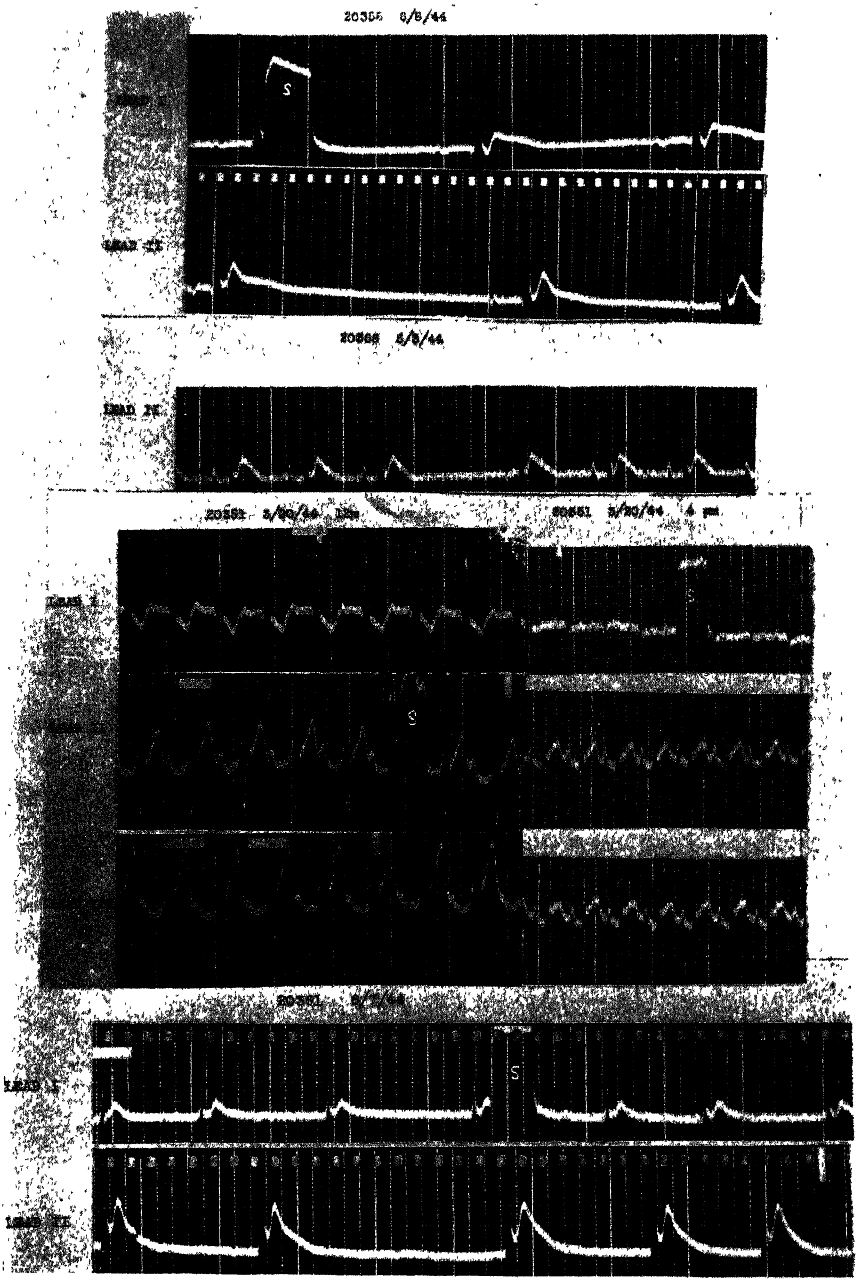

Fig. 2. 20358 third deficiency—first degree A-V block P-R .12- P2,3 are diphasic—QRS is wide (0.018)—heart rate 125. The interval between the light vertical lines is 0.04 sec. in all of these illustrations.

20366 first deficiency—sinus arrest followed by A-V nodal ectopic beat—heart rate 270.

20351 the tracings from this rat were taken after 33 days on 3 micrograms of thiamine daily. Note the bundle branch block (QRS 0.035 sec.) and first degree A-V block (P-R 0.08), bradycardia and T wave changes. The tracings on the right, taken 4 hours later, are normal.

20361 third acute deficiency—auricular fibrillation rhythm completely irregular—no P waves are seen.

This syndrome was not observed in the other rats although it could have been missed easily since it was of such short duration. Similar episodes were not observed in the control rats or in rats on other experiments, although a similar episode was detected in one rat on another thiamine deficiency experiment. In this rat, sectioning of the left vagus nerve almost completely abolished the defect. Because of the low incidence of this abnormality, it has not been possible to study it further.

When thiamine was completely withdrawn after 8 or 10 weeks, the weights began to drop sharply. In an average of 8 days (var. 4 to 19) after complete thiamine withdrawal, acute polyneuropathy occurred. Electrocardiograms were taken on 20 rats during the first, 15 in the second, and 6 in the third acute episode.

As a rule the longer the rats were continued on the thiamine deficient regimen, the more pronounced the electrocardiographic changes became. In some animals the defects were more or less the same in succeeding deficiency episodes, in others the pattern changed from time to time. Disregarding bradycardia, which was found in all rats, electrocardiographic defects were found in 12 of 20 rats in their first, in 14 of 15 in their second, and 6 of 6 in their third period of acute deficiency symptoms.

Tables 1, 2, and 3 summarize the various defects found and their response to thiamine therapy.

Heart rate. The average heart rate in the first, second, and third acute deficiency episodes was 303, 273 and 242 per minute respectively, and after thiamine therapy rose to 431, 422 and 470 per minute, also respectively.

Electrocardiograms taken in the intervals between acute manifestations showed that definite bradycardia (and other changes) was usually present a week or more before the onset of polyneuropathy, but tended to become more and more severe as it developed.

Electrocardiograms taken during the spasticity and convulsions of acute thiamine deficiency would sometimes be so obscured by muscle interference that they were unreadable. However, by taking the tracings in the intervals between convulsions and by calming the rat by means of a cloth thrown over its head, tracings quite free of muscular interference could be secured. In spite of the difficulties, electrocardiograms were taken routinely during this period because it indicated a more or less uniform end-point and also indicated a point beyond which the animal could not become more deficient without dying.

Following thiamine therapy the acute symptoms would disappear in 8 to 12 hours. The heart rate would begin to increase in 4 to 8 hours and in most instances be at normal levels in 24 to 48 hours. In one instance a slow but regular rhythm with a normal sinus mechanism changed into a markedly irregular rhythm with evidence of sinus node damage (20368, table 1) after thiamine therapy. Heart rates below 250 per minute would not respond consistently to thiamine.

Rhythm. Many of the deficient rats showed striking disorders of rhythm. One instance of auricular fibrillation was found, rat 20361, table 3. This rat was in its third period of acute deficiency symptoms and was treated too late to determine whether the fibrillation might have responded to thiamine treatment. Portions of the tracing are shown in figure 2.

Auriculo-ventricular nodal rhythms were found in two rats (20352, 20369A) in their first, and one (20369) in its second acute deficiency. In the two rats

TABLE 1

First acute deficiencies

RAT	DURING DEFICIENCY			AFTER TREATMENT		
	Rate	P-R	Rhythm—other findings	Rate	P-R	Rhythm—other findings
20351	205	0.042	Marked arhythmia, varying sites of stimulus formation, variable P1, 2, 3	430	0.04	Normal
20352	272	0.03–0.035	A-V nodal rhythm, occasional auricular beats	390	0.042	Normal
20354	340	0.05	First degree A-V block, P2, 3, T2, 3 very high, QRS widened (0.019)	450	0.04	QRS 0.015, P2, 3 still high, T2, 3 normal
20355	300	0.053	First degree A-V block, P2, 3 high and widened, QRS widened (0.015)	370	0.04	Normal QRS 0.01
20356	300	0.05	P2, 3 high and wide	500	0.041	Normal
20358	430	0.04	Normal	530	0.038	Normal
20359	333	0.045	Normal	490	0.04	Normal
20359D	333	0.044	Normal	500	0.045	Normal
20361	360	0.05	First degree A-V block	450	0.04	Normal
20363	160	0.04–0.047	Marked irregularity, shifting pace-maker, positive and negative P waves			Died 2 hours after treatment
20364	360	0.045	Normal	430	0.041	Normal
20365	300	0.039–0.043	Marked sinus arhythmia; sinus arrest, auricular ectopic beats	450	0.039	Normal
20366	270	0.053–0.04	Moderate sinus arhythmia, sinus arrest, nodal ectopic beats	450	0.041	Normal
20367	270	0.043	Normal			Died
20368	250	0.043	Moderate sinus arhythmia	260–375	0.03–0.043	Nodal ectopic beats, sinus arrest, arrythmia
20369	320	0.05	First degree A-V block	409	0.041	Normal
20369A	260	0.033	A-V nodal rhythm	475	0.036	Normal
20369B	270	0.047	Normal	425	0.038	Normal
20369C	250	0.047	Normal	375	0.041	Normal
20369D	270	0.055	First degree A-V block, QRS wide (0.015)	480	0.036	Normal QRS 0.01

in their first deficiency, reversion to a normal rhythm occurred after thiamine therapy. The rat in its second deficiency died after treatment.

Sinus arhythmia of some degree was almost always present. In many of the rats this arhythmia was marked and completely irregular. The R-R interval of successive beats would commonly vary by 50 per cent. In addition, ectopic

TABLE 2

Second acute deficiencies

RAT	DURING DEFICIENCY			AFTER TREATMENT		
	Rate	P-R	Rhythm—other findings	Rate	P-R	Rhythm—other findings
20351	200	0.043	Marked arhythmia, shifting pace-maker, ectopic auricular and A-V nodal beats, T waves diphasic	300	0.041	Negative P3
20354	315	0.042	P2, 3 T2, 3 very high	450	0.04	P and T waves still high
20356	300	0.04	P1, 2, 3 inverted, ST2, 3 −4mm.			Died
20358	200	0.01–0.06	Marked arhythmia, QRS wide (0.015) A-V nodal and auricular ectopic	450	0.043	P waves indistinct, variable shape and direction
20359	333	0.053	First degree A-V block	425	0.043	Normal
20359D	310	?	T1, 2, 3, inverted			Killed
20361	214–270	0.051	Sinus arhythmia, sinus arrest auricular and nodal ectopic beats	500	0.038	Normal
20365	273	0.04–0.047	Marked sinus arhythmia, sinus arrest, aur. and nodal ectopic beats	450	0.039	Normal
20368	210	0.061	First degree A-V block, P2+−			Killed
20366	264	0.057	First degree A-V block	430	0.043	Normal
20369	321	0.03	A-V nodal rhythm			Died
20369A	295	0.045	Marked sinus arhythmia T1, 2, 3 inverted			Died
20369C	224	0.033–0.038	Marked sinus arhythmia, variable P waves, nodal ectopic beats	430	0.035	Variable P waves
20369D	200	0.057	First degree A-V block			Killed

TABLE 3

Third acute deficiencies

RAT	DURING DEFICIENCY			AFTER TREATMENT		
	Rate	P-R	Rhythm—other findings	Rate	P-R	Rhythm—other findings
20351	204	0.042	Diphasic T2, 3			Died
20354	315	0.05	First degree A-V block, P2, 3 T2, 3 very high	490	0.04	P and T waves lower
20358	125	0.06–0.12	A-V block, QRS wide (0.018) rhythm irregular, variable P2, 3			Killed
20361	130–200	—	Auricular fibrillation			Died
20365	136–185	0.053–0.057	Moderate sinus arhythmia, P1, 2, 3 variable, A-V block			Killed
20366	290	0.058	A-V block, right and left ventricular ectopic beats			Died

beats of auricular, nodal or ventricular origin would frequently intensify the arhythmia (fig. 2).

Another frequent defect was sinus arrest (fig. 2). Sometimes one, sometimes 3 or 4 A-V nodal or auricular ectopic beats would occur before the sinus mechanism began to function again. These periods of sinus arrest would occur at irregular intervals 2 to 20 times per minute.

P-R interval. Seven of 20 rats in their first, 6 of 15 in their second, and 4 of 6 in their third deficiency episodes had P-R intervals of 0.05 or more indicating a first degree A-V block. Usually these were accompanied by marked variations in the shape, width or direction of the P waves indicating a disturbance in auricular impulse formation and conduction. Figure 2 shows increased P-R intervals. One P-R interval of 0.12 second was observed (fig. 2). In some instances the P-R intervals varied irregularly from beat to beat. As a rule thiamine therapy would return the P-R interval to normal.

Second degree and complete A-V blocks were observed, but only in rats in agonal stages. Since these blocks have been observed also in rats dying of other causes, no significance has been attached to the observation.

QRS interval. In 5 instances significant widening of the QRS complex occurred. In 3 instances this occurred in the first deficiency, promptly returned to normal after therapy and did not recur. In rat 20358, however, QRS increased to 0.015 sec. in its second deficiency, reverted to 0.01 sec. after 50 micrograms of thiamine had been given and then again increased to 0.018 in the third deficiency.

Q T interval. No significant deviations were detected except for those due to changes in heart rate.

P waves. Changes indicating a shift in the pacemaker were fairly common. Abnormally high or wide P waves were found in some rats. In others the P waves were splintered or the form changed from beat to beat. Some records showed P waves varying from positive to negative in the same leads. P wave changes responded slowly and incompletely to thiamine therapy.

T waves. Abnormally high T 2 and 3 were observed in one rat. Diphasic or inverted T 1, 2 and 3 were observed in 3 rats. These T wave changes responded poorly to thiamine treatment.

Pathology. The hearts of 14 of the rats on the thiamine deficient regimen were suitable for histologic examination. Pathologic alteration of the auricular myocardium was found in eight animals; six of these animals also showed involvement of the pulmonary veins. In addition lesions of the pulmonary veins were seen in three rats without cardiac lesions being found. There was no demonstrable involvement of the ventricles. With reference to the incidence of lesions it should be pointed out that only two levels of the heart were studied and previous experience (4) has shown that step-block serial sections would reveal lesions in the hearts of thiamine deficient rats where routine sections had failed to show any pathologic alteration. The pathologic alteration seen in these hearts was similar in all respects to that described in detail by Ashburn and Lowry (4). The lesion is essentially necrosis of muscle fibers followed by cellular infiltration,

fibroblast proliferation and varying degrees of fibrosis. In five hearts the auricular involvement was extensive and bilateral, in two, the lesions were of moderate extent and found only in the left auricle and in one heart single small foci of necrosis were found in both auricles.

DISCUSSION. Although a majority of the rats, which were studied histologically, showed lesions in the auricular myocardium, there was no constant relationship between the pathology and the electrocardiographic changes. A few rats showed electrocardiographic changes without pathology being demonstrated, and one rat showed extensive lesions in the auricles but no electrocardiographic abnormalities.

This situation should not be unexpected since it seems likely that the ability of these lesions to produce electrocardiographic changes would depend on the site as well as the extent of the histologic damage. Furthermore, such definite and severe anatomical changes must be preceded by a period of local metabolic disturbances. It is very probable that these metabolic disturbances could produce cardiac abnormalities without demonstrable histologic evidence.

This concept fits well with the results obtained from treating these thiamine deficient animals with thiamine. One would expect the abnormal metabolic processes to be rather rapidly corrected by the administration of thiamine. Such, apparently, was the case in this experiment since abnormalities, such as bradycardia, sinus arhythmia, and sinus arrest were almost uniformly returned to normal within a few hours after thiamine treatment. Other defects such as the increased P-R and QRS intervals responded quickly at times, and in other instances responded slowly and incompletely to thiamine. Still other changes such as the abnormally shaped and directed P waves responded very poorly or not at all to thiamine. This would be expected if the altered tissue processes had progressed to the point where tissue death had occurred.

The basic pathology of the heart in human thiamine deficiency is not well established. Lesions which might correspond to the focal necrosing lesions seen in experimental animals have been reporedt by Durch (12), Dock (13), Hussey and Katz (14) and Smith and Furth (15). Other reports have been negative or inconclusive (10, 11, 16). It would seem that this subject should be re-examined with especial attention to the auricular myocardium.

SUMMARY

Electrocardiograms from a group of rats chronically deficient in thiamine have been studied.

A symptom complex accompanied by right or left bundle branch block has been described. This lesion occurred in two rats on low thiamine intakes who manifested none of the usual signs of acute thiamine deficiency.

In later stages, when thiamine was withdrawn completely, a variety of electrocardiographic abnormalities were encountered. These changes developed a week or more before polyneuropathy occurred and usually became more and more severe until treatment was given. The following changes were noted: (1) bradycardia, (2) sinus arhythmia, (3) auricular fibrillation, (4) A-V nodal rhythms,

(5) sinus arrest, (6) shifting pacemaker, (7) first degree A-V block, (8) auricular, A-V nodal and ventricular ectopic beats, (9) widened QRS, (10) P and T wave changes. In most instances these defects were partially or completely corrected after thiamine therapy.

In a considerable percentage of these rats there were areas of pathological changes in the auricular myocardium and pulmonary veins.

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EFFECT OF DESTROYING THREE LOCALIZED CEREBRAL CORTICAL AREAS FOR SOUND ON CORRECT CONDITIONED DIFFERENTIAL RESPONSES OF THE DOG'S FORELEG[1,2]

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This fourth report on the function of the association cells of the cerebral cortex of the dog is concerned with the effects of removal of the acoustic projection areas on correct conditioned differential foreleg reflexes. These areas, 3 in number (fig. 1), are those from which Tunturi obtained voltage changes by tonal and by electrical stimulation of localized regions of the cochlea. The two largest areas were previously described in the cat by Woolsey and Walzl from localized electrical stimulation of the cochlea. It has been proved by these investigations and assumed from the publications of Fletcher, Steinberg and Lipman that the space pattern for tones is conducted in anatomical units to the cerebral cortex. As in my previous work the emphasis is to be placed on the correctness of the motor response rather than on fine tonal discriminations.

The general procedure, including the controls used, has been given in an earlier paper. Extraneous sound and sight were eliminated. Two different conditioned differential sound tests were used. In the first, based on tones, a bell and iron cup tapped once per second served for the positive and negative conditioned reflex stimuli; while in the second, based on timing, a bell tapped once per second and three times per second functioned for the two opposing stimuli. To be correct the positive conditioned reflex had to appear within 7 seconds and the negative conditioned reflex had to last for 10 to 15 seconds, depending on the time interval of the positive response.

The following tests were made to determine whether these dogs were basing their responses on differences of tones. The fundamental tones of the bell and cup were found to be about 2600 and 720 vibrations respectively. A number of normal and operated dogs which had acquired correct conditioned differential responses for the bell and cup analysers were afterward tested with these pure tones from a loud speaker and all responded correctly during their first tests. Loudness was eliminated as a factor by varying occasionally the intensity of the stimuli in a positive or negative test or in the same test, but ordinarily an attempt was made to keep the stimuli of equal strength.

To be selected for these experiments a dog must acquire the positive conditioned reflex during the first 25 trials and correct conditioned differential responses in 2 sessions. A daily session consisted of 37 tests, of which 25 were with the positive conditioned reflex and 12 with the negative conditioned reflex, with an occasional reversal of this order. The first and majority of daily sessions

[1] Aided by a grant from the John and Mary R. Markle Foundation.

[2] The anesthetic, vinbarbital sodium, was donated by Sharp and Dohme.

consisted of a series of 5 positive tests to 2 negative tests up to and including the 20th positive test, then 5 negative tests, 4 positive, 1 negative and 1 positive.

To diminish situational tension during the tests which can lead to tetany, convulsions or neurosis the writer always maintained a very friendly relationship to the dogs by attending to their feeding, etc.

Complete elimination of the cortex concerned, including the deep sulci, was accomplished with minimum damage to the white matter by extirpation and coagulation. The area to be extirpated was first circumscribed by a blade possessing a depth gauge. It was then undercut with a spatula and usually removed in one piece. Coagulation was accomplished by a series of 15 second exposures of a 5 mm. ball electrode supplied by a Wyeth high frequency surgical unit.[3] If the field was in proper condition the second method produced a uniform burn to the depth of the white matter. With both methods the optic radiations, which are on a level with the median portions of the posterior ectosylvian gyrus, were damaged to the extent of blindness.

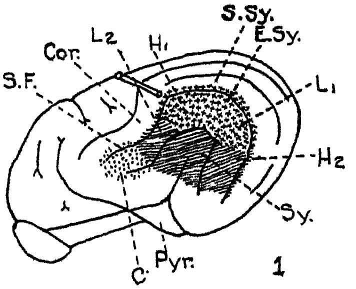
Elimination of Cortical Areas A, B or C (fig. 1). Area A was extirpated or coagulated in 3 dogs, area B in 2 dogs and area C in 3 dogs (one not tabulated). Formalin preparations showed destruction confined to these areas, involving all of the gray and some of the superficial white matter, but in no instance did the injury include the brain stem. Marchi stained sections through the medulla revealed only normal degeneration in the pyramids. There were no motor symptoms and the foreleg placing reflexes were strong. All of the dogs responded normally to spoken words. The lesions involving area A produced blindness, but these dogs could go up and down stairs rapidly by tapping nose on the steps.

Bell and cup and slow and fast bell analysers. Since tables 1 and 2 demonstrate that destruction of any of these areas produced no apparent effect on previously learned correct conditioned differential responses for either set of sound analysers it suffices to record only the following: Before operation all of the dogs acquired correct conditioned differential responses with either set of analysers during the 1st or 2nd sessions and one or two additional sessions resulted in perfect records. Following the unilateral lesions the 1st session of tests resulted in perfect or practically perfect scores for both sets of analysers. The bilateral lesions produced no effect on the early appearance or character of the positive conditioned reflex. After the bilateral lesions, column 3 of tables 1 and 2 discloses the presence of correct conditioned differential responses during the 1st sessions of tests with both sets of analysers for all of the dogs, excepting dog 1. The delayed appearance of correct responses in this dog may be due to a more extensive involvement of the suprasylvian gyri. The perfect records for daily sessions shown in columns 4 and 5 were obtained not later than the 4th session in dogs 2 to 7, and the records for all tests (columns 6 and 7) demonstrate little or no difficulty in reacquiring correct conditioned differential responses.

After the lesions all of the dogs were able to respond correctly to alternated positive and negative conditioned reflex tests for both sets of analysers when the interval between tests was of 3 seconds' duration.

[3] Tests with 15 and 10 second exposures of the 5 mm. electrode on the surface of liver produced uniform destruction to depths of 8 and 4.5 mm. respectively.

Elimination of Cortical Areas A and B. Coagulation was used for dogs 8 and 9 and extirpation for dogs 10 to 13. Formalin preparations of the brains of dogs 8, 9, 10 and 12 show destruction limited to areas A and B, which includes strips of the coronal and suprasylvian, as well as the entire sylvian and ectosylvian gyri. At the time of the left side operation, the sulci of dog 11 appeared irregular and the brain (fig. 2) reveals small cephalic and caudal portions of areas A and B present and apparently uninjured. The extirpations of dog 13 (fig. 3) not only included areas 1 and 2, but extended on both sides into area 3. For the most



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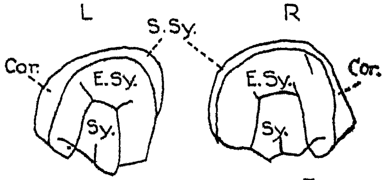

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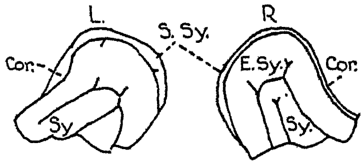

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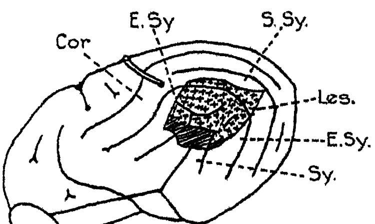

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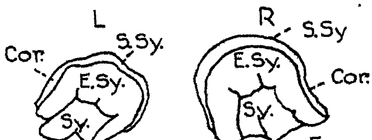

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Fig. 1. Dog's brain showing cortical areas destroyed by lesions; area A, crosses; area B, parallel lines; area C, dots.

Fig. 2. Incomplete removal of areas A and B on left side of dog 11.

Fig. 3. Extirpations from dog 13.

Fig. 4. Extirpations from dog 18.

Fig. 5. Extirpations from dog 19.

Abbreviations: *L* and *R*, left and right side; *C*, area C; *Cor.*, coronal gyrus; *E.Sy.*, ectosylvian gyrus; H_1, high tone region area A; H_2, high tone region area B; L_1, low tone region area A; L_2, low tone region area B; *Les.*, lesion; *Pyr.*, pyriform area; *S.F.*, suprasylvian sulcus; *S.Sy.*, suprasylvian gyrus; *Sy.*, sylvian gyrus.

part the white matter appeared to be in good condition and in no instance was the brain stem injured.

Of six dogs used, four were trained and two were not trained before the operations. They included easily excited, easily inhibited and the neutral types of cerebration. As a result of the lesions all of the dogs were blind. Their foreleg placing reflexes were strong. There were no motor symptoms. Marchi sections through the medulla revealed no conspicuous degeneration in the pyramids. Several paid no attention to spoken words.

Bell and cup analysers. Column 2 of table 1 shows that dogs 8, 9 and 10

TABLE 1

Analysers: bell and cup

CORTICAL AREA	BEFORE OPER. DIFF. FIRST APPEARED SESSION NO.	AFTER OPER. DIFF. FIRST APPEARED SESSION NO.	DAILY SESSIONS AFTER DIFF. STANDARDIZED		TOTAL NO. OF TESTS		CHARACTER OF CORRECT CON. DIFF. RESPONSES
			Pos. C to I	Neg. I to C	Pos. C to I	Neg. I to C	
A							
Dog 1, E.........	2d	4th	25-0 11-1	1-12 1-24	159-4	43-51	good
Dog 2, C.........	2d	1st	25-0	1-11	68-3	3-27	good
Dog 3, C.........	2d	1st	25-0 12-0	0-12 0-25	90-7	0-61	good
B							
Dog 4, E.........	2d	1st	25-0 12-0	0-12 0-25	84-4	6-65	good
Dog 5, E	1st	1st	25-0 12-0	0-12 1-24	57-6	5-44	good
C							
Dog 6, E..	1st	1st	25-0 12-0	0-12 1-24	37-0	1-36	good
Dog 7, E	2d	1st	25-0 12-0	0-12 0-25	50-0	3-59	good
A and B							
Dog 8, C.........	1st	13th	25-0 13-0	0-13 0-25	338-34	62-121	good
Dog 9, C.........	2d	10th	25-0 12-0	0-13 0-25	263-34	95-53	good
Dog 10, C........	1st	11th	25-0 12-0	0-12 0-25	358-4	101-94	good
Dog 11, E*.......	2d	2d*	25-0 12-0	1-13 0-25	61-1	6-45	good
Dog 12, E.... ...	Oper. before testing	11th	24-1 12-0	0-12 2-23	296-66	75-109	good
Dog 13, E†.......	Oper. before testing	27th†	25-0 12-0	1-13 2-23	580-235	275-245	good
Later............		None 30	25-0 13-0	12-0 25-0	678-3	448-0	no

* Extirpation incomplete on left side for cortical areas A and B.

† Extirpation extended into region of cortical area C on both sides.

Abbreviations used in the tables

Diff. = Correct conditioned differential responses; Pos. = Positive conditioned reflex; Neg. = Negative conditioned reflex; Oper. = Operations; No. = Number; E. = Extirpated; C. = Coagulated; C to I = ratio of correct to incorrect responses; I to C = Ratio

(good lesions) acquired correct conditioned differential responses during the 1st and 2nd sessions and perfect records were obtained in 1 or 2 additional sessions. Unilateral lesions permitted correct conditioned differential responses immediately. Bilateral lesions produced no effect on the positive conditioned reflex, but greatly delayed the appearance of correct conditioned differential responses. Column 3 shows absence of correct responses until the 13th session (471 tests) for dog 8, the 10th session (270 tests) for dog 9 and the 11th session (407 tests) for dog 10. The perfect records for columns 4 and 5 required 185 to 276 additional tests. Columns 6 and 7 demonstrate the difficulties each dog experienced with the positive and negative conditioned reflexes. In contrast dog 11 (incomplete lesion on left side) is shown by table 1 to have produced correct conditioned differential responses during the 2nd session and perfect scores during the 3rd and 4th sessions.

As for the two dogs not trained until after the bilateral lesions had been made, dog 12 in which the lesion was well placed, exhibited no signs of correct conditioned responses (table 1, column 3) until the 11th session (407 tests). The good showings of columns 4 and 5 were the records of the 14th and 15th sessions. Dog 13 is of special interest since the lesion (fig. 3) in addition to including areas A and B extends over into area C on both sides, representing a transition between this group and the following group in which the lesions involved all three cortical areas. Column 3 of table 1 discloses that, following the lesions, no signs of correct conditioned reflexes appeared until the 27th session (1000 tests), but ultimately good records were obtained for a daily session (columns 4 and 5). The positive conditioned reflex, however, appeared during the first session with the 8th test. Six months later after this dog had demonstrated inability to make correct conditioned differential responses for the slow and fast bell and for 2 conditioned differential tests with general cutaneous sense, dog 13 was retested with the bell and cup analysers. The results of these tests are recorded directly below the earlier tests in table 1. The complete absence of correct conditioned differential responses for 30 sessions (1110 tests) is obvious from columns 3, 4 and 5 of the table. Column 7 shows that 448 negative conditioned tests resulted in 448 positive responses in spite of an equal number of punishments.

Slow and fast bell analysers. The 4 dogs trained before the lesions were made acquired correct conditioned differential responses during the 1st or 2nd sessions and perfect records on or before the 4th session. Unilateral lesions permitted correct responses immediately. As shown by column 3 of table 2 correct conditioned differential responses did not occur for dogs 8, 9 and 10 after the bilateral lesions until the 5th, 8th and 12th sessions. This delay in appearance of the correct responses, though somewhat less than recorded for the previous analysers, is significant because these dogs were able to make perfect scores for the bell and cup tests before the slow and fast bell tests were started. The perfect records of columns 4 and 5 were for the 14th and 15th sessions for dog 8, the 11th and 12th sessions for dog 9 and the 22nd and 23rd sessions for dog 10. The difficulties encountered by these dogs for making correct responses are clearly revealed by columns 6 and 7. Dog 11 (incomplete lesion on left side)

TABLE 2

Analysers: slow and fast bell

CORTICAL AREA	BEFORE OPER. DIFF. FIRST APPEARED SESSION NO.	AFTER OPER. DIFF. FIRST APPEARED SESSION NO.	DAILY SESSIONS AFTER DIFF. WAS STANDARDIZED		TOTAL NO. OF TESTS		CHARACTER OF CORRECT CON. DIFF. RESPONSE
			Pos. C to I	Neg. I to C	Pos. C to I	Neg. I to C	
A							
Dog 1, E	1st	4th	25-0 12-0	0-12 0-25	171-0	38-95	good
Dog 2, C	2d	1st	12-0 12-0	0-6 0-12	24-0	0-18	good
Dog 3, C	1st	1st	25-0 12-0	0-12 0-25	113-1	4-48	good
B							
Dog 4, E	1st	1st	25-0 12-0	0-12 0-25	120-4	16-106	good
Dog 5, E	1st	1st	25-0 12-0	0-12 1-24	222-15	36-99	good
C							
Dog 6, E	1st	1st	13-0 12-0	0-6 0-25	25-0	0-31	good
Dog 7, E	1st	1st	25-0 12-0	0-12 0-25	37-0	1-36	good
A and B							
Dog 8, C	1st	5th	25-0 12-0	0-12 0-25	152-10	44-55	good
Dog 9, C	1st	8th	24-1 12-0	0-12 0-25	211-11	60-41	good
Dog 10, E	2d	12th	25-0 12-0	1-11 0-25	482-3	166-167	good
Dog 11, E*	2d	3d*	24-1 12-0	0-11 0-25	83-4	10-41	good
Dog 12, E	Oper. before testing	7th	25-0 12-0	0-12 0-25	238-10	80-77	good
Dog 13, E†	Oper. before testing	None 30†	25-0 14-1	12-0 23-2	692-12	440-29	no

* Extirpation incomplete on left side for cortical areas A and B.
† Extirpation extended into region of cortical area C on both sides.
For list of abbreviations used in table see table 1.

is shown by column 3 of table 2 to have only slight delay in the return of correct conditioned differential responses.

As for the untrained dogs—table 2 reveals that dog 12 (lesion correct) encountered about the same difficulties of acquiring correct conditioned differential responses as were recorded for dogs 8, 9 and 10, while dog 13 (lesions extend into area 3 both sides) furnished no correct responses for 30 sessions involving 1110 tests.

All of the dogs which could make correct conditioned differential responses for both sets of sound tests were also able to make correct responses to the alternate positive and negative conditioned tests when the interval between tests was of 3 seconds' duration.

Elimination of Cortical Areas B and C. Coagulation was used for dog 14 and extirpation for dogs 15 and 16. There was no damage to area 1 in any dog. However, not all of the posterior portion of area 2 was removed from the left side of dog 15. This region, however, does not include the cortex from which sound frequencies corresponding to the fundamental tones of the bell and cup have been recorded. All of the dogs appeared to be normal in every respect with the exception of slight motor disturbances in hind legs of dog 16. Marchi stained sections did not disclose any noteworthy degeneration in the pyramids.

Bell and cup analysers. According to table 3, column 2, dogs 14, 15 and 16 acquired correct conditioned differential responses with the first session of tests. After the unilateral lesion correct responses appeared immediately. Following the bilateral lesions the positive conditioned reflex appeared promptly and not modified, but column 3 of table 3 reveals some irregularity in the time of appearance of the correct conditioned differential responses. With dog 14 there were signs of correct responses during the 1st and 16th sessions, but none in the interval between. With dog 15 (incomplete lesion on one side) and dog 16 the first correct responses came with the 6th and 2nd sessions. The good results for a session of tests shown in columns 4 and 5 were the results of the 22nd and 23rd sessions for dog 14, the 10th and 11th sessions for dog 15 and the 6th and 8th sessions for dog 16. The total record of columns 6 and 7 shows some difficulty with the negative conditioned reflexes.

Slow and fast bell analysers. Table 4, column 2, discloses that only one session was required for learning correct conditioned differential responses. After the unilateral lesion correct responses appeared with the first tests. Following the bilateral lesions, column 3 of table 4 shows correct conditioned differential responses for dog 14 during the 4th session, and during the 2nd and 3rd sessions for dogs 15 and 16. The perfect records of columns 4 and 5 were from the 6th and 7th sessions for dog 14, the 3rd and 4th for dog 15 and the 9th and 10th for dog 16. Column 7 discloses very little difficulty in regaining the negative conditioned reflex.

After the bilateral lesions all of the dogs were able to make correct responses for a series of alternate positive and negative conditioned tests when the interval between tests was of 3 seconds' duration.

Six months after the operations and following similar tests to the above with

general cutaneous sense, dogs 14 and 16 were retested with the bell and cup and the fast and slow bell analysers. Both dogs demonstrated signs of correct conditioned differential responses with the first session of tests for each set of analysers, but it required 2 to 4 additional sessions before perfect control was established for the negative conditioned reflexes.

TABLE 3

Analysers: bell and cup

CORTICAL AREA	BEFORE OPER. DIFF. FIRST APPEARED SESSION NO.	AFTER OPER. DIFF. FIRST APPEARED SESSION NO.	DAILY SESSIONS AFTER DIFF. WAS STANDARDIZED		TOTAL NUMBER OF TESTS		CHARACTER OF CORRECT CON. DIFF. RESPONSE
			Pos. C to I	Neg. I to C	Pos. C to I	Neg. I to C	
B and C							
Dog 14, C	1st	1st or 16th?	25-0 12-0	0-12 0-25	580-11	218-111	good
Dog 15, E	1st	6th	25-0 13-0	1-11 1-24	236-2	74-65	good
Dog 16, E	1st	2d	25-0 13-0	0-12 2-23	177-11	22-89	good
A, B & C							
Dog 17, C	2d	none 31	25-0 12-0	14-0 25-0	717-25	464-22	no
Dog 18, E	1st	none 30	25-0 12-0	12-0 25-0	727-3	392-0	no
Dog 19, E*	1st	none 30*	25-0 13-0	12-0 25-0	677-24	404-7	no
Dog 20, E	1st	none 30	25-0 12-0	13-0 25-0	722-3	421-2	no
Dog 21, C† 10 sec.	1st	8th†	25-0 12-0	0-12 1-24	230-20	69-89	good

* Extirpation included very little of cortical area C on left side.

† Results from superficial coagulation.

For list of abbreviations used see table 1.

Elimination of Cortical Areas A, B and C. These areas (fig. 1), which included all of the sylvian and ectosylvian gyri together with neighboring strips of the coronal and suprasylvian gyri, were extirpated in dogs 18, 19 and 20, and coagulated for 15 seconds with dog 17 and more superficially for 10 seconds with dog 21. Formalin prepared brains demonstrated satisfactory lesions for dogs 17, 18 and 20. A drawing (fig. 4) of the extirpations from dog 18 is typical of the others. Figure 5 shows absence of area 3 in the left side extirpation of dog 19; while

only the outer half of the cortex of dog 21 was destroyed by the superficial coagulation. Marchi stained sections of the medullae showed no pronounced degeneration in the pyramids.

There were no motor symptoms. The foreleg placing reflexes and the positive general cutaneous conditioned foreleg reflex were present. All were blind, but

TABLE 4

Analysers: slow and fast bell

CORTICAL AREA	BEFORE OPER. DIFF. FIRST APPEARED SESSION NO.	AFTER OPER. DIFF. FIRST APPEARED SESSION NO.	DAILY SESSIONS AFTER DIFF. WAS STANDARDIZED		TOTAL NUMBER OF TESTS		CHARACTER OF CORRECT CON. DIFF. RESPONSE
			Pos. C to I	Neg. I to C	Pos. C to I	Neg. I to C	
B and C							
Dog 14, C	1st	4th	25-0 12-0	0-12 0-25	162-2	48-56	good
Dog 15, E	1st	2d	25-0 12-0	0-12 0-25	81-6	5-56	good
Dog 16, E	1st	3d	20-0 13-0	0-12 1-24	183-5	30-118	good
A, B & C							
Dog 17, C	1st	none 31	25-0 15-0	13-0 25-0	728-5	438-9	no
Dog 18, E	2d	none 30	25-0 12-0	13-0 13-0	698-2	413-6	no
Dog 19, E*	2d	7th*	25-0 12-0	0-12 1-24	121-65	28-121	good
Dog 20, E	2d	none 30	25-0 13-0	12-0 25-0	711-3	390-0	no
Dog 21, C† 10 sec	1st	4th†	25-0 12-0	0-12 1-24	136-1	37-48	good

* Extirpation included very little of cortical area C on left side.

† Results from superficial coagulation.

For list of abbreviations used see table 1.

could go up and down stairs rapidly by tapping nose on the steps. Dogs 17 to 20 paid no attention to spoken words.

Bell and cup analysers. According to table 3, column 2, all of the dogs acquired correct conditioned differential responses during the 1st or 2nd daily sessions and perfect records for the 2nd, 3rd or 4th sessions. Unilateral lesions permitted practically perfect scores for the first session of differential tests. With the exception of dog 18, none of the dogs had any difficulty with the posi-

tive conditioned reflex after the bilateral lesions. This dog, however, was able from the start to make only a very generalized response involving all four legs, and required 28 sessions (700 tests) before the response could be confined to one leg. When localized the response was very strong and positive. After the bilateral lesions column 3 of table 3 reveals that dogs 17, 18, 19 (area 3 present on left side) and 20 never reacquired correct conditioned differential responses during 30 sessions (1100 tests). The records for the last two sessions (columns 4 and 5, table 3) and the total tally (columns 6 and 7) show the same proportion of positive responses for the negative conditioned tests as for the positive tests. Occasionally, however, after much punishment for a long series of negative conditioned errors, a negative test and the following positive test will result in absence of foreleg response, *incorrect inhibition.* A few punishments for positive conditioned errors will usually start all tests going positive again.

On the other hand, dog 21, in which the coagulation of areas A, B and C was purposely made superficial on both sides, demonstrated correct conditioned differential responses during the 8th session (300 tests) after the lesions. The perfect scores of columns 4 and 5 were for the 9th and 10th sessions.

Six months later, following the slow and fast bell tests and 2 different general cutaneous sense tests, dogs 17, 18, 19 and 20 were still unable to make correct conditioned differential responses for the bell and cup. On the other hand dog 21 (superficial coagulation) produced perfect differential records after the 8th session.

Slow and fast bell analysers. These tests followed the preceding and on the same dogs. All of the dogs acquired correct conditioned differential responses before the lesions during the 1st or 2nd sessions and perfect records in one or two additional sessions. Unilateral lesions caused no delay in the appearance of correct responses. Following the bilateral lesions none of the dogs with satisfactory lesions (17, 18 and 20) were able to make correct conditioned differential responses during 30 sessions (1100 tests). Columns 4 and 5 of table 4, which contain the records for the 29th and 30th sessions and columns 6 and 7, which include the total score, conclusively show that practically all of the positive and negative conditioned tests resulted in positive foreleg responses. On the other hand, it is revealed from column 3 of table 4, that dog 19 (incomplete lesion on left side) and dog 21 (superficial coagulation) produced correct conditioned differential responses during the 7th and 4th sessions. This, however, represents many more tests than were required for the learning period before the operations. Excellent later records for dogs 19 and 21 are shown in columns 4 and 5.

Seven months after the lesions dogs 17, 18 and 20 were still unable to make correct conditioned differential responses with the slow and fast bell, while dogs 19 and 21 ultimately scored perfect records. Dog 19, however, was still unable to make correct responses with the bell and cup analysers.

Dogs 19 and 21 were also capable of making correct responses to a series of alternated positive and negative conditioned tests when the interval between tests was of 3 second duration.

Discussion. Practically all studies on the effects of cortical lesions on hearing were made before and consequently without the aid of the recent localization investigations of Woolsey and Walzl on the cat and Tunturi on the dog. Much of the interest has centered on ability to make fine discriminations of sound. Many of the lesions have been concerned with removal of Munk's area, which includes the caudal half of areas A and B of figure 1 and the posterior arm of the suprasylvian gyrus. Its removal was said by Munk to produce psychic deafness in dogs. Luciani and Seppilli's dog S in which all of the caudal halves of the cerebrum were removed with the exception of area 3 on one side was said to have lost all perception of sound. Kalischer found that a dog previously trained to pick up meat for a certain sound and leave it alone for another sound could repeat the act after removal of Munk's areas. Rothmann in a better set of tests trained a dog to come to him at the sound of a certain note and to lie down for another note. Retention of these responses followed removal of the sylvian gyri.

As reported by Eliasson and Pavlov, Babkin established two different meat salivary reflexes to two different tones and this discrimination was possible after removal of Munk's areas. However, when two conditioned reflexes were established using the same tones, but with different sequences of time between the tones, removal of Munk's areas prevented the establishment of this differentiation. In addition, Babkin trained a dog named "Ruslau" to discriminate with the salivary reflex between ascending and descending tones. After removal of Munk's area not only was this discrimination lost, but the dog failed to respond to his name. Eliasson established two different conditioned reflexes to two different tones and also to a folk song in which So14 appeared and another to the same song in which So14 was absent. After removing two-thirds of the anterior portion of Munk's areas this differentiation ultimately reappeared. In one dog Eliasson was able to inhibit a salivary sound reflex with 5 or 6 trials, but upon removal of Munk's areas this inhibition was not elicited during 61 trials.

Following removal of the sylvian gyri Girden reports complete loss of left and right side sound discrimination, while other large temporal lobe lesions simply depressed it. Pennington on the other hand maintains that the amount of left and right side discrimination is dependent on the amount of cerebral cortex destroyed. Klüver and Bucy have reported auditory agnosia symptoms in apes following temporal lobe lesions. A quotation from Pavlov's lectures as translated by Gantt states that ablation of the posterior half of the cerebral cortex leaves a blind and deaf dog, while complete decerebration leaves a helpless idiot.

The difference in results from the writer's lesions involving cortical areas A and B, and A, B and C, namely, delay in appearance and prevention of correct conditioned differential responses with auditory stimuli, suggests that only a small portion of the association cortex may be required for these responses. This is in part agreement with Lashley's conclusions on discrimination of visual patterns. It was essential, however, that this cortex include an area from which tonal differentiation can be recorded.

It was shown previously (1940, 1941 and 1943) that frontal lobe association

cells were not important for correct conditioned differential responses with auditory and general cutaneous stimuli. However, removal of the prefrontal or pyriform-amygdaloid areas abolished correct conditioned differential responses with olfactory stimuli for 200 to 400 tests;[4] while much larger lesions of the posterior association cortex than those of cortical areas A, B and C produced no effect on correct conditioned differential responses with olfactory stimuli.

The exact damage to the cortical association mechanism for sound from the largest lesions is unknown. It could include all of it or it could comprise: 1. Terminal endings of the auditory projection fibers. 2. Association cells concerned with correlation of sound or of associated memory. These cells might relay directly to the foreleg motor cortex to bring about correct flexion of the foreleg or to the cells of the suppressor bands of Dusser de Barenne which have connections that might be capable of producing absence of foreleg response for the negative conditioned reflex. 3. The suppressor band cells might be destroyed. The position of these bands have not been determined for the dog, but in the cat Garol places one of these bands in the region of area C and another in the suprasylvian gyrus. Tower has located a large extrapyramidal inhibitory area in the sylvian and ectosylvian gyri of the cat. Lack of *correct inhibition* was apparently the chief impediment of all dogs unable to make correct conditioned differential responses as a result of the lesions, while *incorrect inhibition* appeared occasionally. 4. Severance of important interconnecting association circuits between the cortex, corpus striatum and thalamus may have been a disturbing factor to the timing mechanism necessary for maintenance of correct conditioned differential responses. The presence of positive conditioned reflexes immediately after all lesions indicated the presence of sound impulses in the cortex and the functioning of the motor cortex.

The great number of tests required for re-establishment of correct conditioned differential sound responses after bilateral destruction of cortical areas A and B suggest actual damage to the usual sound association circuits and the re-establishment of new circuits. This is supported by the fact that after a dog reacquires correct conditioned differential responses with the bell and cup analysers it takes about the same large number of tests to reacquire correct responses with the slow and fast bell. The fact that the previously untrained dogs had as much difficulty as the previously trained dogs in acquiring correct conditioned differential responses after the lesions suggests importance of areas A and B as localities for the formation of sound association circuits.

This reacquired sound association mechanism, which was as accurate as the original, was apparently lacking in other respects. In agreement with Eliasson's observations for the salivary conditioned reflexes, some of our operated dogs

[4] Some records of this study suggest that more olfactory tests would have resulted in the establishment of new association circuits from which correct olfactory conditioned differential reflexes could be elicited. This, however, is not indicated from a recent report (This Journal **139:** 553, 1943) from which it was shown from oscillographic records that electrical stimulation of the pyriform lobe resulted only in voltage changes in the frontal association cortex and from an area, which, in the cat, is concerned with inhibition of the electrical activity of the motor cortex.

lost the ability to make correct conditioned differential responses much earlier than normal dogs. A number of the dogs while being able to make perfect responses with the new association circuits did so under more tension as exhibited by tremor, fast or deep breathing and more general excitation.

The timing mechanism of Lorente de Nó involving interconnecting neurons should lend itself very nicely to the procedure of formation of the original and the reacquired conditioned reflex circuits.

A comparison of the records of the effects of the lesions on cortical areas B and C with those of the other areas in which correct conditioned differential responses were re-established after the lesions would seem to place this group closer to the groups in which the lesions involved A, B or C than to the group involving areas A and B.

In general the positive conditioned reflex was but little affected by the lesions. In some instances it might be said to be a little stronger, weaker or more generalized, but this response has variations in a normal dog.

SUMMARY AND CONCLUSIONS

Deletion of cortical areas A, B, C or B and C on both sides caused but little or no delay in appearance of previously acquired correct conditioned differential responses of the foreleg with two different sets of sound stimuli.

Bilateral elimination of cortical areas A and B disrupts the usual association circuits for evoking correct conditioned differential responses for two different sets of sound analysers. Re-establishment of these responses required 370 to 484 tests. The new mechanism was fully as accurate as the original, but was inferior in other respects.

Unilateral elimination of cortical areas A, B and C permitted immediate return of correct conditioned differential responses with both sets of sound analysers.

Bilateral destruction of areas A, B and C caused no noteworthy delay in the appearance of the positive conditioned reflex.

Complete bilateral destruction of cortical areas A, B and C (sylvian, ectosylvian and strips of the coronal and suprasylvian gyri) in 3 dogs prevented reappearance of correct conditioned differential responses in over 1100 tests for each dog with each set of analysers and for additional tests 6 months later. The presence of area 3 on the left side of one dog in which this operation was attempted, permitted correct responses for the slow and fast bell stimuli, but abolished all correct responses for the bell and cup. Superficial coagulation of areas A, B and C in one dog resulted only in a delayed return of the correct responses.

The first and chief effect noted from the larger lesions was one involving correct inhibition, inability to hold back foreleg flexion during a negative conditioned test.

While the usual time interval between tests was about 2 minutes, all of the dogs which were able to make correct conditioned differential responses after the lesions were also able to respond correctly to a series of alternate positive and negative conditioned tests when the interval between tests was of 3 seconds' duration.

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LOCAL FLUID LOSS IN TRAUMA[1]

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The loss of fluid from the circulation is one of the major physiological changes in traumatic shock. In 1919 Cannon and Bayliss expressed a belief that there was not sufficient swelling in the traumatized area to account for the effects of the trauma. This belief, although it was not based on experimental measurement, lent weight to the view that in trauma there is generalized increase in capillary permeability, and consequently, a general loss of fluid from the circulation. In recent years other investigators have subjected this theory of generalized fluid loss to experimental testing. Blalock (1930, 1931), Parsons and Phemister (1930), Cullen and Freeman (1941), Ashworth, Jester and Guy (1944) and Prinzmetal et al. (1944) compared the volume of a traumatized hind limb with that of an untraumatized hind limb by dissection after death. Manery and Solandt (1943) estimated the swelling of traumatized hind limbs by immersing them in water to a mark on the limbs and measuring the water displaced from a full tank. This method is in error if there is considerable spread of swelling up the abdominal wall. Furthermore, except in the work of Ashworth et al. (1944) these experiments did not involve blood volume measurements before and after trauma, so that the relationship between leg volume change and the blood volume change is of the nature of an estimate.

The present work was undertaken by the author in 1943 to discover the relationship between the blood volume change and the local fluid loss in trauma, and constitutes one aspect of the investigations of traumatic shock initiated in this laboratory by Dr. Magnus I. Gregersen.

PROCEDURE. A precise measurement of the local fluid loss was obtained by the use of the apparatus illustrated in figure 1. This tank is shaped to fit the dog board in such a way that frequent measurements can be made with the dog on the board.

The hair is clipped from the dog's back and tail and, by means of plaster of Paris, the waist band *W* and the tail splint *T* are fastened to the dog, the waist-band being attached to the back just above the crests of the iliac. When the plaster has set, the hind end of the dog with the waist-band, tail splint and connecting strip *S*, is slipped into the tank and a water tight seal is provided by a layer of tacky grease between the matching curvatures of *W* and *G*. The tail splint is then fastened by means of bolts to the bottom of the tank, thus firmly

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Columbia University.

and accurately securing the dog within the tank. The cover C is now bolted in place on its gasket R, and the sliding door D gently pressed down upon the dog's belly in such a manner that the sponge rubber gasket SR completes the water tight seal. This portion of the seal is also aided by the liberal application of the tacky grease. The foot end of the dog board is now tilted up $1\frac{1}{2}$ inches to insure complete filling, and water at body temperature is poured into the filling tube F until the tank is filled and water appears in the overflow tubes O,O. The plug P is removed and the water allowed to flow into a collecting pail for weighing. In order to insure uniform and complete drainage, the head end of the dog board is raised $1\frac{1}{2}$ inches above the foot end during the collection of water.

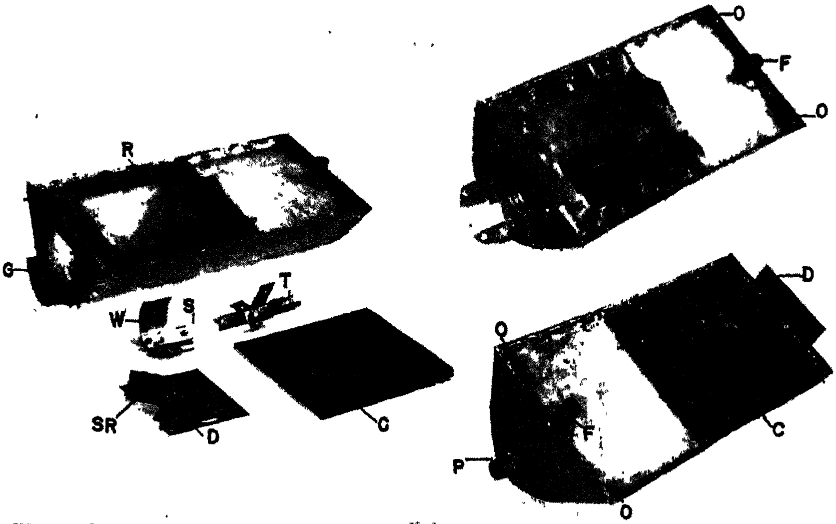

Fig. 1. Several views of the tank used in determining the swelling of traumatized hind limbs is shown. This illustrates separately, and also in combination, the waist band W, the tail-splint T and the connecting strip S; the sliding door D with its sponge rubber gasket SR, the cover C and its gasket R, are also shown. The filling tube F, the overflow tubes O,O and the emptying spout P, are pictured here.

The general experimental procedure was as follows: a set of control measurements of heart rate, blood pressure by arterial puncture, hematocrit, plasma protein by the refractometer, and rectal temperature were taken. During this period the blood volume was determined by use of the dye T-1824 (Gregersen and Stewart, 1939; Gregersen and Rawson, 1943). Following the blood volume measurement, the splints were plastered to the dog, the animal inserted in the tank and several measurements were made of the water drained from the tank. Frequently the animal was shifted and relocated within the tank in order to test the accuracy with which it could be replaced in the original position in the tank. The animal was anesthetized with ether, removed from the tank, and traumatized according to the method of Gregersen and Root (1942). After obtaining blood pressure and heart rate, the animal was carefully relocated in the tank, and the

drained volume again obtained. The difference between the drained volumes measured before and after trauma constitutes a measure of the swelling of the traumatized limbs. Subsequently the swelling was remeasured at intervals of about 90 minutes. The heart rate, blood pressure, hematocrit, plasma protein and rectal temperature changes were observed throughout the remainder of the experiment. At least one determination of the blood volume by the dye method was made after trauma.

The pressure of the gasket, *SR*, on the dog's abdomen does not measurably affect the blood pressure, the hematocrit, or the plasma protein values. However, this gasket was applied only during the measurement of the drainage volume, a duration of about five minutes for two determinations. The cover *C*

TABLE 1

DOG NO.	DRAINAGE			DRAINAGE AFTER RESETTING			DIFFERENCE OF MEANS
	Grams	Mean	Mean dev.	Grams	Mean	Mean dev.	
1	12780			12795			
	12830			12805			
	12800	12803	18	12795	12798	5	5
2	12780			12810			
	12800	12790	10	12810	12810	0	20
6	13510			13510			
	13535	13523	13	13505	13508	3	15
16	12485			12490			
	12500	12493	8	12530			
				12500	12507	16	14
Average difference of the means							14
Average of mean deviations							9

was also removed between drainage measurements thereby making the femoral vessels easily accessible.

Results. The accuracy of the method for determining swelling is illustrated by the data in table 1. The drainage values are given (4 dogs) for successive fillings of the tank at one position. After the dog had been shifted from the tank and replaced as closely as possible in its original position, the drainage values were again obtained. It was concluded that the probable error in replacing the animal and in each drainage measurement was less than 15 cc.

Figure 2 illustrates a typical experiment. The first measurement of limb swelling was accomplished 24 minutes after the beginning of trauma and showed an increase of 333 cc. in the volume of the limbs. One hour later the dye (T-1824) was injected intravenously. Blood samples were taken during the next hour for the blood volume determination. The difference between the blood volume measured before, and the value obtained after the trauma, is 270 cc.

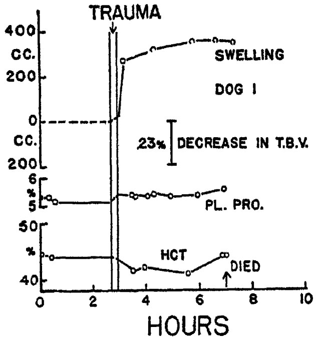

Fig. 2

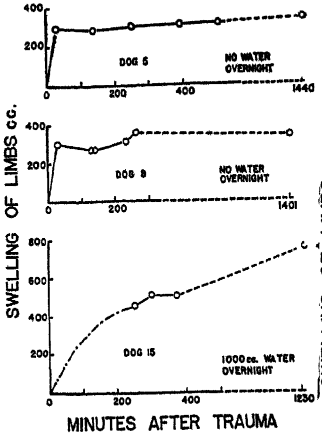

Fig. 3

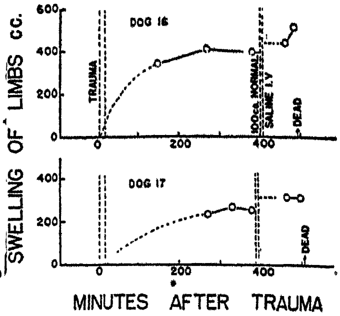

Fig. 4

Fig. 2. This illustrates a characteristic record of a trauma experiment showing the values of the hematocrit, the plasma proteins, the swelling of the limbs and the decrease in the total blood volume after trauma.

Fig. 3. Showing the limb swelling in three dogs which survived overnight. Dogs 5 and 8 were given no water and showed no significant swelling overnight. Dog 15 drank a liter of water and had a large overnight limb swelling.

Fig. 4. This shows the limb swelling appearing in two cases after the injection of 100 cc. of 0.9 per cent saline intravenously.

TABLE 2

DOG	WEIGHT	BLOOD VOLUME			LIMB SWELLING				LIMB SWELLING AT DEATH		SURVIVAL TIME
		Before trauma	After trauma	Change A	Min. after trauma	Volume B	Excess C	Just after trauma D	cc.	cc./kgm. E	
	kgm.	*cc.*	*cc.*	*cc.*		*cc.*	%	*cc./kgm.*			*min.*
1	7.00	826	635	191	24	270	+41	39	350	45	265
2	7.51	759	516	243	74	300	+24	40	325	43	334
3	8.47	945	682	263	40	420	+59	50	—	—	treated
4	8.00	995	490	505	35	527	+4	66	525	66	140
5	9.55	930	744	186	30	297	+60	31	—	—	survival
6	4.43	509	396	113	31	155	+37	35	210	47	160
7	8.55	861	626	235	50	320	+36	37	383	45	252
8	9.40	788	598	190	30	300	+58	32	—	—	survival
9	4.82	486	435	51	10	65	+27	14	135	28	272
10	9.21	969	703	266	30	333	+25	36	415	45	195
11	5.91	628	503	125	35	210	+68	36	240	41	long duration
12	9.64	913	611	302	30	334	+11	35	415	43	255
13	7.95	761	358	403	23	450	+11	57	420	53	90
14	7.15	762	418	344	30	342	−1	48	—	—	treatment
Average values (all animals).....................							+33	39			
Per cent of body weight (average)..............								4.1			
Average value (nonsurvival animals)............										46	
Per cent of body weight (average)..............										4.8	

Column C gives the per cent excess of the swelling of the limbs soon after trauma above the change in blood volume. This is given by the equation $C\% = 100\ (B - A)/A$.

Columns D and E give the swelling of the limbs in terms of cubic centimeters per kilo-

Thus the swelling of the limbs shortly after trauma exceeds the change in blood volume by 41 per cent. Table 2 gives the percentage excess of the swelling of the traumatized region above the change in blood volume in 14 experiments. These values which range from −1 per cent to +68 per cent have an average of +33 per cent. Therefore, the swelling of the traumatized region is adequate to account for the blood lost from the circulation.

Following the first measurement of swelling after trauma, the volume of the traumatized region continued to increase for a few hours. In cases where the survival was of sufficient duration, the volume approached a limiting value. This limit is probably set by the balance between the hydrostatic pressure of the bloodstream, the osmotic pressure (largely due to the plasma proteins in the blood) and the tissue pressure in the limbs (Youmans et al., 1934). It will be noted in table 2 that the experiments designated as "survival" or "long duration" have a relatively larger initial swelling than those of shorter duration. The blood pressure in the experiments of long duration is at a higher level than the blood pressure in the shorter experiments and will tend to produce a greater leakage into the tissues. The blood volume after trauma remains relatively constant in the untreated fatal cases according to the findings of Gregersen and Root (1942). Therefore, it appears that the continued swelling of the traumatized region is due to the transfer of water and electrolytes from the interstitial space via the circulation to the extravascular traumatized areas.

The extent of the swelling immediately following trauma and also at the time of death is shown in table 2. The mean values are 39 and 46 cc. per kgm. of body weight respectively. If the fluid causing the swelling in the limbs is assumed to have the specific gravity of blood, i.e., 1.05, it is easy to compute the swelling as a percentage of body weight. The values become 4.1 per cent of the body weight immediately following trauma and 4.8 per cent at death.

Several of these experiments were conducted with certain modifications which necessitate their separate description. The first group (fig. 3) are survival animals. These dogs were placed in their cages overnight and relocated in the tank on the following day. Dog 15 drank one liter of water during the night. On the following day, 15 hours after the previous measurement of leg volume, an additional swelling of 245 cc. was found. For comparison dogs 5 and 8 were given no water overnight and showed insignificant leg volume changes, i.e., an increase of 21 and a decrease of 25 cc. respectively in more than fifteen hours. These results suggest that after the first six hours following trauma, the subsequent swelling of the traumatized regions is slight unless fluid is given to the animal.

The effect of an intravenous injection of isotonic saline is demonstrated in figure 4. Both dogs 16 and 17 were given intravenously 100 cc. of saline at the times indicated on the charts. The values of the swelling, which had attained a steady level before the injection, showed increases of 100 cc. and 50 cc. respectively, shortly after the injection.

Dog 14 (fig. 5) demonstrates the effect of a transfusion of whole blood upon the swelling of the traumatized region. This swelling (150 min. after trauma) had about reached its constant value, and since the animal was failing rapidly,

an intravenous transfusion of 300 cc. of whole blood was given during the next fifteen minutes. The volume of this transfusion almost equaled the measured

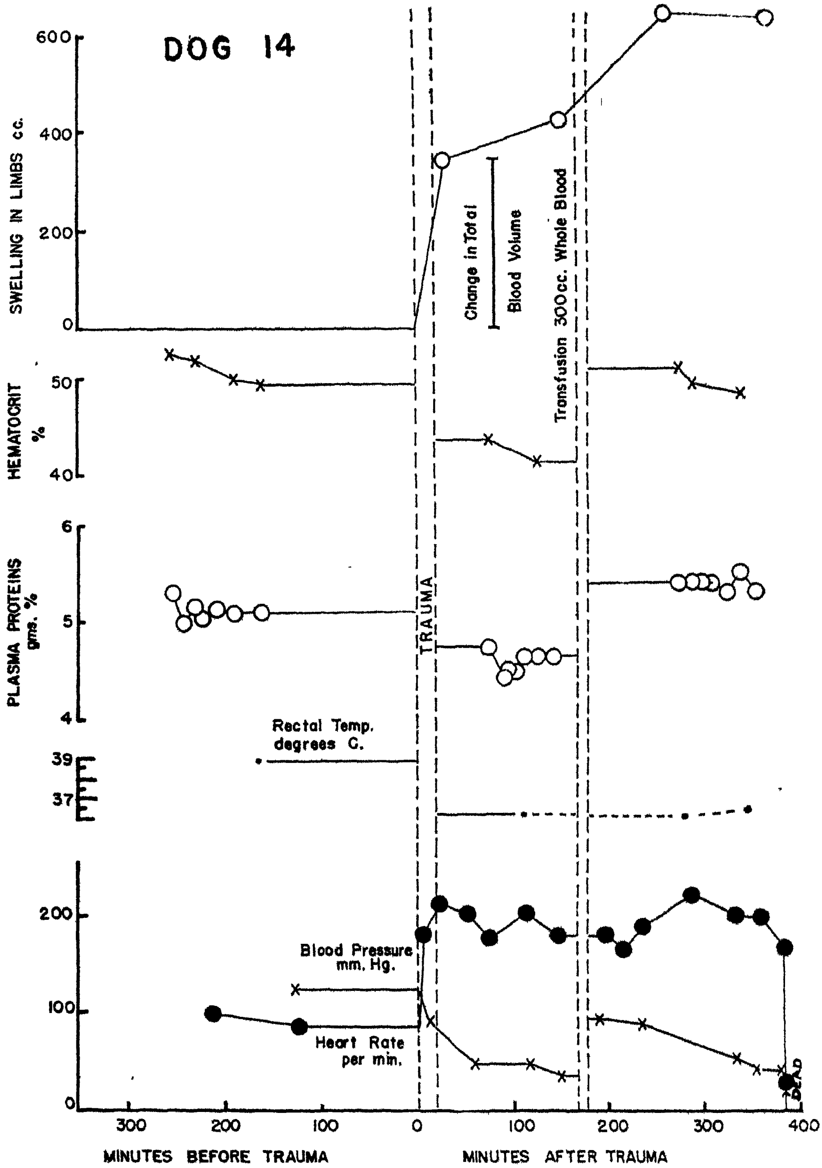

Fig. 5. Showing the effect of trauma followed by a transfusion of whole blood upon limb swelling, hematocrit, plasma protein, rectal temperature, blood pressure and heart rate.

decrease of the blood volume which was 344 cc. Following the transfusion the blood pressure rose to 90 mm. As a result of this disturbance in the fluid pressure equilibrium, an additional swelling of 217 cc. appeared in the traumatized

region. In this experiment the transfusion was not adequate to save the animal and the falling blood pressure prevented further swelling of the limbs.

A review of the fluid shifts involved in this experiment is of some interest. At the time of transfusion the animal had 240 cc. of plasma, 178 cc. of cells, and 9.3 grams of plasma protein. The transfusion added 170 cc. of plasma, 130 cc. of cells and 11.0 grams of protein. The total amounts then become 410 cc. of plasma, 308 cc. of cells and 20.3 grams of plasma protein. A blood volume determination started 97 minutes after the transfusion, showed that 263 cc. of plasma, 254 cc. of cells and 14.2 grams of protein were present in the circulation. This means that the loss from the circulation, presumably into the traumatized region, was 147 cc. of plasma, 54 cc. of cells and 6.1 grams of protein. The volume of fluid lost from the circulation was 201 cc., an amount very close to the measured increase in swelling of the limbs (217 cc.). These results and those of the preceding experiments are not without interest in a consideration of the fate of some of the transfusion fluids.

Discussion. In comparing the results obtained in these experiments with the findings of other workers, two situations should be considered. First, the experiments in which dissection at death is the method by which the swelling of the traumatized region is determined give terminal values (Blalock, 1930, 1931; Parsons and Phemister, 1930; and Cullen and Freeman, 1941). From the preceding papers one can obtain data on 8, 5, 6 and 6 dogs respectively. These results show the mean limb swellings in percentage of total body weight to be 5.3, 3.8, 4.3 and 4.6 correspondingly. The weighted mean of these values is 4.6 per cent of the body weight. The above values compare favorably with the value obtained here (4.8) on 10 nonsurviving animals and gives support to the method presented in this paper. The data of Ashworth et al. (1944) give the relationship between limb swelling and the change in plasma volume. Their estimate for total blood lost into the limbs is 4 per cent of the total body weight, a lower value than above since the group of subjects included some possible survival animals.

The second method of measurement, immersion to a mark on the limbs, will give results which tend to be low inasmuch as swelling up the abdominal wall is not measured. The work of Manery and Solandt (1943) furnishes the values of 2.2 just after trauma and 3.3 per cent of body weight at death for the nonsurvival animals. These results are considerably lower than the corresponding values of 4.1 and 4.8 obtained in the present work, the differences being probably accounted for by the method of immersion in which the swelling up the abdomen was not measured.

The question of the adequacy of the swelling of the limbs to account for the change in the blood volume after trauma appears to be answered satisfactorily by the data presented here. In no experiment was the first measurement of swelling following trauma significantly less than the change in blood volume.

SUMMARY

1. A method was devised for the measurement of the volume of swelling appearing in the traumatized limbs of the dog.

2. Measurements of the swelling appearing within one hour after trauma showed an average swelling of 33 per cent in excess of the change in total blood volume (14 dogs). This swelling was adequate to account for the measured reduction in blood volume.

3. The swelling in untreated animals tends towards a maximum limit a few hours after trauma. The addition of fluid to the animal either by the absorption of water from the gut or by intravenous saline, plasma, or whole blood is accompanied by a further increase in swelling in the traumatized area.

4. In fatal untreated cases the average swelling just after trauma was 4.1 and at death 4.8 per cent of the total body weight.

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PHOSPHATE TURNOVER IN MUSCLE DURING SHOCK[1]

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The changes that occur in muscle after the application of a tourniquet which completely occludes the blood supply are similar to those of autolyzing muscle (1). Phosphocreatine is almost completely hydrolyzed in one hour. Adenosine triphosphate and glycogen almost disappear after three hours. The inorganic phosphates and lactic acid of the muscle increase to a maximum in about four hours. If the flow of blood is restored to the muscle within three hours there is resynthesis of phosphocreatine and adenosine triphosphate with a corresponding decrease of the inorganic phosphate. Fatal shock does not develop even though large amounts of muscle have been occluded. If the occlusion is released after more than three hours there is no regeneration of the organic phosphates and considerable inorganic phosphate is washed from the injured muscle into the blood. Fatal shock develops in rats so treated if sufficient muscle has been occluded but there is no evidence to suggest that inorganic phosphate or the hydrolytic products of the organic phosphates of muscle are important agents in the causation of shock.

The fact that phosphates are washed out of injured muscle suggests that there is increased permeability of the injured muscle. Fine, Seligman and Frank (2) tagged plasma proteins with isotopes S^{35}, Br^{82} and I^{133} and found that the tagged plasma proteins escaped into the region of injury in considerable amounts but not into untraumatized regions. Engel and Forrai (3) studied the excretion of acid fuchsin into the knee joint of cats. When the same leg was crushed there was an increased excretion of the dye but when the forelegs were crushed there was no change.

Corcoran, Taylor and Page (4) observed a marked depression of renal function in dogs soon after the application of partially occluding tourniquets to the hind limbs of anesthetized dogs. They ascribed this depression to a humoral vasoconstricting substance which could be demonstrated in the plasma of such animals. There was a marked decrease of the diodrast clearance, which was usually greater than the decreased inulin clearance. On the basis that flow of renal plasma to the functioning excreting tissue was measured by the diodrast clearance and the rate of formation of glomerular filtrate from renal plasma was measured by inulin clearance, the depression of renal function appeared to be due to reduction of renal blood flow. After release of the tourniquets there was a depression of the diodrast extraction ratio so that a change of equilibrium between plasma and renal interstitial fluid was postulated. This change may have been due to arteriovenous shunting or to altered permeability of the renal epithelium.

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It seemed to us that a study of the transfer of phosphates from the blood to the muscle would be of interest for several reasons. It would indicate whether the membranes of injured muscle can maintain normal control of the transfer of phosphates from the blood and whether any correlation existed between the permeability of the muscle and its ability to regenerate the organic phosphates. Since the viability of muscle is associated with the ability to regenerate these phosphates and since the occurrence of shock is somewhat dependent on the viability of the muscle, it might be possible to correlate the occurrence of shock with a change in the permeability of the injured muscle. Information on any change in permeability of the muscle not injured directly by application of the tourniquet would also be of value.

Preliminary experiments indicated that the transfer of phosphates from the blood to the muscle could be measured one hour after intravenous injection of radioactive sodium phosphate by determining the amount of phosphorus in the plasma and its radioactivity and the radioactivity of the phosphorus of the muscle. Seven groups of rats were studied in this way. The first group was normal anesthetized rats from which blood and the muscle from both hind legs were taken for analysis one hour after administration of P^{32}. In group two, a rubber band occluded the blood supply to one hind leg for one hour and was released at the time of injection of P^{32}; one hour later blood and the previously occluded muscle and the muscle of the opposite leg were taken for separate analysis. In group three both forelegs and one hind leg were occluded for one hour and then released and P^{32} was given; one hour later blood and the muscle of both hind legs were taken for separate analysis. In group four, one hind leg was occluded for four hours with subsequent treatment similar to that of the other groups after release of the occlusion. In group five, one hind leg was occluded for three and a half hours and released for one hour before P^{32} was given, blood and muscle being taken for analysis one hour later. In group six, both forelegs and one hind leg were occluded for four hours and released at the time of injection of P^{32}; samples were taken for analysis one hour later. In group seven both forelegs and one hind leg were occluded for three and a half hours and released for one hour before the injection of P^{32}; samples were taken for analysis one hour later. In this way studies were made of the rate of transfer of phosphate from the blood to the uninjured muscle and the previously injured muscle in groups of rats representing normal animals, animals with mild muscle injury but no signs of shock, animals with muscle injury and early signs of shock and animals with muscle injury and severe shock.

Procedure. Male rats weighing from 200 to 250 grams were anesthetized with pentobarbital sodium. A rubber band was wrapped tightly about the thigh and moved to the upper part of the thigh with the aid of a hemostat acting as a lever. In all cases there was sufficient pressure to overcome the arterial pressure. At the times indicated the constricting bands were cut and dibasic sodium phosphate containing 20 microcuries of radioactive phosphorus from the Berkeley cyclotron and approximately 0.6 mgm. of phosphorus was injected intravenously. A few minutes prior to obtaining specimens for analysis

the skin was loosened from the legs. Blood was withdrawn into a heparinized syringe by cardiac puncture exactly one hour after administration of the radioactive phosphorus. The Achilles tendon was severed and pulled upward and the flexor muscles of the leg were severed with one stroke and dropped immediately into a mixture of solid carbon dioxide and alcohol. The frozen muscle was powdered between cooled steel blocks and transferred frozen to tared tubes containing ice-cold 5 per cent solution of trichloracetic acid and glass beads. The tubes were shaken in a room at 0°C. for fifteen minutes and the solution was filtered.

The inorganic phosphate of the muscle was precipitated at once from this extract as the magnesium ammonium salt. After standing four hours in the cold room, it was filtered, washed and dissolved in hot 5 per cent nitric acid. The concentration of inorganic phosphate was determined by the method of Fiske and Subbarow (5) and the radioactivity was measured with a scale-of-four, Geiger-Müller counter of the immersion type (6). The filtrate and washings from the magnesium ammonium phosphate were combined, made to volume and analyzed for concentration and radioactivity of the organic phosphate. The water content of the muscle was determined by drying the muscle to constant weight at 110°C. The plasma was extracted with trichloracetic acid and analyzed for P and P^{32} content. All measurements of radioactivity were corrected for decay and recorded as counts per second per milligram of phosphorus.

Results. The phosphate entering the muscle from the plasma one hour after being labeled with radioactive phosphorus was compared in seven groups of rats. The previous treatment of each group is indicated in table 1. Other rats not included in this study were allowed to recover from their anesthesia after the removal of the constricting band around the legs. Animals similar to groups 6 and 7 were usually in a state of collapse at the time the specimens would have been taken and the blood pressure was too low for accurate readings. Several animals in the groups 6 and 7 intended for muscle analysis died before the end of the one hour period after receiving P^{32}. All of the animals similar to groups 1 to 5 were in good condition with no appreciable fall of blood pressure one hour after the release of the occluded legs. All animals similar to groups 2 and 3 made complete recoveries and regained the normal use and appearance of the occluded legs after the edema had subsided (two or three days). Those treated like groups 4 and 5 recovered but there was considerable necrosis of the occluded muscle only a few regained adequate function in the injured leg after three or four weeks and the muscle mass did not become comparable to that of the uninjured leg.

The results of the experiments are summarized in tables 1, 2 and 3. A marked increase of the plasma inorganic phosphate occurred in groups 4, 5, 6 and 7, which corresponds to the severity of experimental procedure. Hemoconcentration had occurred in each of these groups as indicated by hematocrit values of 50.8 ± 1.7, 56.1 ± 1.3, 52.6 ± 0.6 and 62.6 ± 0.9 respectively. The hematocrit values of groups 1, 2 and 3 were 40.5 ± 1.1. There was considerable variation of the specific activity of the plasma inorganic phosphate within each group

but there were no significant differences between the various groups except that groups 1 and 3 had lower specific activities because less P^{32} had been administered.

There was a definite increase of the amount of inorganic phosphates in the muscles of the unoccluded leg of groups 2, 4, 5, 6 and 7. This increase was accompanied by a relative decrease of the amount of organic phosphate present in the corresponding muscle. These changes may be associated with the factors producing shock but we have frequently observed similar changes in experiments involving muscular activity and some manipulation of rats anesthetized for more than one hour. The fact that blood was withdrawn by cardiac puncture immediately before removal of the muscles may also have been a greater factor in the rats with more extensive experimental procedures. The specific activity of the inorganic phosphates varied considerably in the different groups. It was definitely less in comparison with the specific activity of the plasma in the experimental groups than in the normal animals. The specific activity of the organic phosphates of the muscle also varied considerably but was from 36 to 20 per cent of the specific activity of the inorganic phosphate of the same muscle.

The marked increase of the inorganic phosphate of the occluded muscle of groups 4, 5, 6 and 7 was due to the failure of resynthesis of the organic phosphates after the release of the occlusion, as is evident by the low values obtained for the corresponding organic phosphates. Most of the inorganic phosphate in group 5 had been washed out of the muscle by the blood during the two hour period after the release of the occlusion. The specific activity of the inorganic phosphate was definitely greater in the previously occluded muscle than in the unoccluded muscle of groups 2, 3, 4 and 5 but not in groups 6 and 7. The specific activity of the organic phosphates of the muscle was from 34 to 18 per cent of that of the inorganic phosphates.

To obtain an index of the permeability of the muscle to phosphates from the plasma the following calculations were made: The total radioactivity of the inorganic and organic phosphates of the muscle expressed as total counts each second was divided by the specific activity of the inorganic phosphate of the plasma. The result is the number of milligrams of phosphorus in the muscle that have the same radioactivity as that of the plasma. For example, the inorganic phosphates of muscle of unoccluded leg of group 1 averaged 26.5 mgm. per 100 grams of muscle with 100 counts for each milligram or a total of 2,650 counts. The organic count on the same basis was 4,211, and the total inorganic and organic count was 6,861. The muscle was 76.4 per cent water and 100 grams of dry muscle would contain 29,060 counts. Each milligram of phosphorus in the plasma contained 1,033 counts and 100 grams of dry muscle contained the equivalent of 28.1 mgm. of phosphorus with the same activity as that of the plasma. The numbers given in table 1 were obtained from the data of each individual experiment and may differ slightly from the calculations based on the average of the same data. Figure 1 shows the amount of phosphorus from plasma in 100 grams of dry muscle in each group and the relative permeability

TABLE 1

Phosphates of plasma and muscle one hour after administration of P^{32}

GROUP	RATS	LEGS OCCLUDED	HOURS MUSCLES WERE OCCLUDED AND RELEASED	PLASMA INORGANIC PHOSPHATE		LABELED PHOSPHATE FROM PLASMA MGM. P IN 100 GRAMS OF DRY MUSCLE	
				Mgm. P in 100 cc.	Specific activity*	Unoccluded	Occluded
1	22	0	0	6.3 ±0.1†	1,033‡ ±36	29 ±1	
2	12	1	1-1	7.3 ±0.2	2,576 ±326	17 ±1	28 ±2
3	9	3	1-1	6.9 ±0.1	1,192‡ ±32	13 ±1	24 ±2
4	13	1	4-1	12.1 ±0.4	2,807 ±266	24 ±2	49 ±5
5	12	1	3½-2	14.7 ±0.4	2,507 ±565	28 ±4	65 ±7
6	12	3	4-1	16.8 ±1.4	3,311 ±457	24 ±3	47 ±5
7	10	3	3½-2	26.6 ±2.7	2,621 +±279	25 ±4	39 ±9

* Specific activity is the number of counts recorded by the Geiger counter each second for each milligram of phosphorus in the corresponding sample.

† The figures after the mark ± represent standard error of the mean.

‡ Animals of these groups received a third as much P^{32} as those of the other groups.

TABLE 2

Phosphates of muscle of unoccluded leg one hour after administration of P^{32}

GROUP	WATER	INORGANIC PHOSPHATES		ORGANIC PHOSPHATES	
		Mgm. P in 100 grams	Specific activity	Mgm. P in 100 grams	Specific activity
	per cent				
1	76.4 ±0.7	26.5 ±1.0	100* ±4	127.6 ±2.5	33 ±1
2	75.7 ±0.6	40.5 ±2.2	135 ±19	115.8 ±4.4	39 ±7
3	77.2 ±0.5	22.2 ±1.1	76* ±5	123.7 ±3.2	14 ±2
4	74.9 ±0.6	42.4 ±1.9	206 ±19	117.7 ±2.6	74 ±7
5	76.1 ±0.5	42.5 ±2.4	171 ±31	109.1 ±4.0	47 ±11
6	74.1 ±1.0	46.4 ±1.7	230 ±21	121.5 ±4.1	72 ±8
7	74.6 ±0.8	46.3 ±3.7	238 ±43	105.6 ±5.0	58 ±14

* Animals of these groups received a third as much P^{32} intravenously as was given to those of the other groups. The relative distribution of P^{32} is similar to that of the other groups.

TABLE 3

Phosphates of muscle of occluded leg one hour after administration of P^{32}

GROUP	WATER	INORGANIC PHOSPHATES		ORGANIC PHOSPHATES	
		Mgm. P in 100 grams	Specific activity	Mgm. P in 100 grams	Specific activity
	per cent				
2	77.8 ±0.5	34.3 ±2.2	217 ±10	107.5 ±2.8	74 ±6
3	77.1 ±0.4	18.4 ±1.5	122* ±7	124.3 ±7.4	34 ±3
4	82.4 ±0.8	76.2 ±3.6	294 ±32	18.8 ±2.6	88 ±11
5	82.6 ±1.1	46.1 ±5.0	544 ±115	23.7 ±5.5	98 ±16
6	80.7 ±1.2	88.7 ±3.2	238 ±34	32.5 ±8.2	85 ±11
7	81.6 ±0.7	77.9 ±6.9	172 ±45	20.3 ±4.8	54 ±7

* Animals of this group received a third as much P^{32} as those of the other groups.

of each in curves obtained by dividing the amount of phosphorus from plasma in the muscle by the amount of phosphorus in the plasma.

COMMENT. The greater uptake of P^{32} by muscle after release of occlusion may be in part due to the inclusion of highly active phosphates from the plasma in the extracellular edema fluid of such muscles. We do not have sufficient information to permit an accurate analysis of the distribution of the inorganic phosphates of the muscle but what information we have indicates that the major increase of radioactive phosphate is not in the edema fluid. In groups 2 and 3

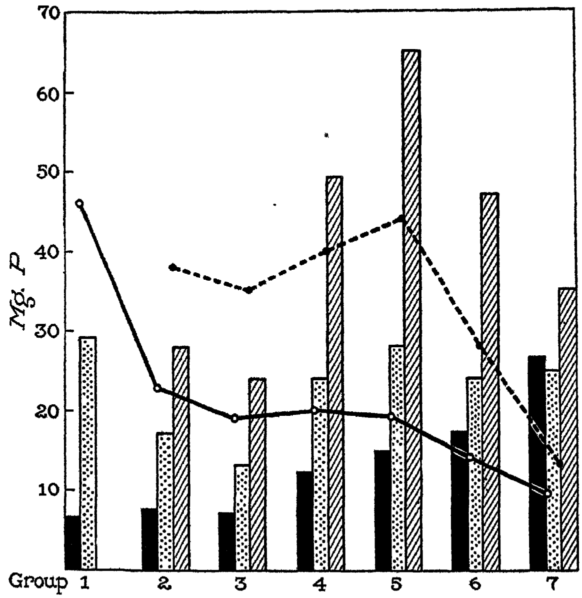

FIG. 1. The transfer of phosphates from the plasma to the muscle. The solid rectangles indicate the average number of milligrams of inorganic phosphate P in 100 cc. of plasma. The stippled rectangles indicate the number of milligrams of P which have entered 100 grams dry muscle of the unoccluded leg in the last hour of the experiment in each group. The crosshatched rectangles indicate the number of milligrams of P which have entered 100 gram dry muscle of the previously occluded leg in the last hour of the experiment. The circles connected with the solid line represent the relative permeability of the unoccluded muscle. The circles connected with the broken line represent the relative permeability of the previously occluded muscle. The assigned figure for relative permeability was obtained by dividing the number of milligrams of P which had entered 100 grams dry muscle by the number of milligrams of P in 100 cc. of plasma.

in which the organic phosphates have approached normal values after release of the occlusion, a large part of the increased radioactivity is in the organic fraction. In the other groups the organic phosphates have been largely hydrolyzed and not resynthesized and the major increase of radioactivity is found in the inorganic fraction. The ratio of the specific activity of the organic phosphates to that of the inorganic phosphates was of the same magnitude in all groups, a fact which should indicate that no large accumulation of P^{32} was sequestered in the edema fluid of the muscle.

In the calculation of the amount of labeled phosphate in the muscle from the plasma certain factors have not been taken into account. The figures obtained represent only the amount of phosphate which is equivalent to the phosphate of the plasma one hour after administration of P^{32}. Certainly the phosphate entering the muscle from the plasma during the early part of the hour had much greater specific activity than that found at the end of one hour. The specific activity of the phosphate diffusing out of the muscle at the same time must have been much lower than that found at the end of the hour. During the hour after receiving P^{32} there was diffusion of phosphates from the blood to the extracellular fluid and thence to the intracellular phosphates and conversion to organic phosphates. At the same time diffusion was equal in the opposite direction to the blood. Since we have no measure of these factors we cannot measure the actual amount of phosphate that entered or left the muscle during the hour. The amount of labeled phosphate present in comparison with that of the blood gives only an index of the total amount that has been transferred. This index may be taken as the relative permeability of the muscle to phosphates although there is no indication whether the change of permeability has been in the capillaries or the muscle itself.

SUMMARY

The amount of radioactive phosphorus present in the muscle one hour after administration of sodium phosphate containing P^{32} is greater in muscle which has been previously occluded for one hour than in the unoccluded muscle of the same anesthetized white rat. Larger amounts are present under similar circumstances in muscles which have been previously occluded for three and a half or four hours. Less P^{32} is present in the unoccluded muscle of rats after release of occlusion of other muscle than is found in control anesthetized rats one hour after administration of P^{32}. If the rise in the phosphates of plasma which accompanies release of previously occluded muscle is taken into consideration it is evident that there is a marked depression of the permeability of unoccluded muscle to phosphates after release of occlusion to other muscle. After signs of shock are present there is a further reduction of phosphate transfer. On the basis of the amount of phosphates present in the plasma, the permeability of muscle after release of occlusion is more nearly that of muscle of normal animals but the transfer of phosphates falls rapidly when signs of shock are present.

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HEMOLYTIC ANEMIA PRODUCED BY THE FEEDING OF FAT AND CHOLINE[1]

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Hemolytic depression of the erythrocyte numbers in dogs accompanied by increases in icteric indices following the oral administration of 10 mgm. per kgm. of choline chloride and 60 grams of lard daily has been reported previously by us (1). Loewy et al. (2) have presented evidence that a high fat diet fed to dogs increases their bile pigment output. The hemolytic agents from fat are presumably soaps and fatty acids which have escaped resynthesis into neutral fat during absorption (3, 4).

The purpose of the present investigation was to learn whether the feeding of 2 doses daily of fat and choline would produce a more marked depression of the red cell count than one dose; and to study the effect of a single dose of each substance on the blood of man.

PROCEDURE. Normal dogs were kept on a basal diet of Purina Dog Chow meal and rolled oats. This diet has maintained dogs in apparently good health for at least 3 years in this labotarory. Red blood cell counts, hemoglobin percentage (Hellige) and readings of the icteric index were made on blood samples drawn from the saphenous veins of the dogs. Samples were drawn only when the animals were unexcited and in a resting, fairly basal, condition—at least 16 hours after previous feeding or medication. Reticulocyte percentages were determined on films of blood smeared with brilliant cresyl blue solution and counterstained with Wright's stain.

After normal control values had been determined on the blood of 3 dogs, choline and fat feeding was commenced. Each animal was given 60 grams of "Vegetole"[2] twice daily and 10 mgm. of choline chloride per kgm. of body weight twice daily. The latter was administered by stomach tube in dilute solution.

Two human subjects were given at one meal (breakfast) a supplement of $\frac{1}{4}$ pound of butter and 400 mgm. of choline chloride. The blood was studied at intervals before and after this procedure.

RESULTS. Figure 1 shows the effect of the administration of 2 doses daily of fat and choline to 3 normal dogs upon their red blood cell counts. Depressions of 20 to 37 per cent in the erythrocyte numbers were produced by this procedure. Hemoglobin percentages were reduced, reticulocyte percentages were not significantly changed, but significant elevations of the icterus indices did occur (table 1). After 4 days, the fat feeding was discontinued but choline administration was not interrupted, and the erythrocyte numbers showed a tendency to rise during the next 2 days.

[1] Research paper No. 567, Journal Series, University of Arkansas.

[2] Armour's shortening made from animal and vegetable fats.

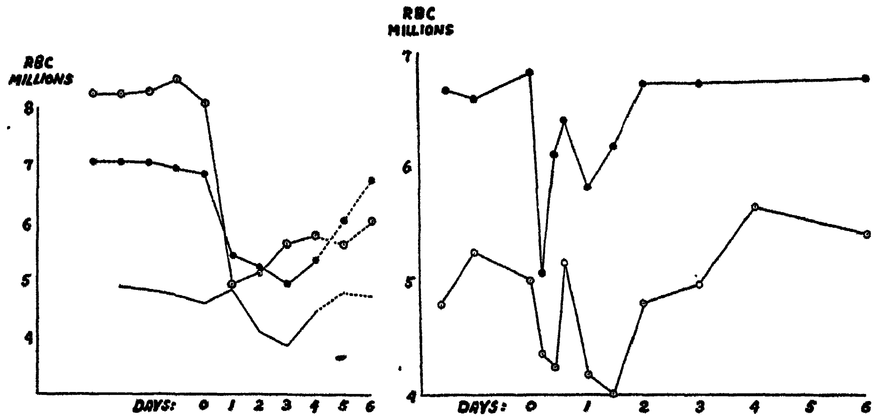

Fig. 1. Red blood cell counts on 3 dogs which received fat and choline twice daily for four days beginning at zero time.

Fig. 2. The effect of a single dose of choline and fat upon the erythrocyte numbers of two human subjects.

TABLE 1

Effect of fat and choline feeding on the blood of 3 normal dogs

	HEMOGLOBIN PERCENTAGE (100% = 14.5 GRAMS)	RETICULOCYTES	ICTERIC INDEX
	per cent	*per cent*	
Dog 1 Normal	79	0.2	6.6
Choline and fat 1st day	79		24.0
2nd day	68		20.0
3rd day	71	0.1	
4th day	69		
5th day	75	0.2	
Dog 2 Normal	110	0.1	9.5
Choline and fat 1st day	93		25.0
2nd day	90		22.8
3rd day	98	0.2	
4th day	98		
5th day	100	0.05	
Dog 3 Normal	103	0.1	8.0
1st exper. day	88		15.0
2nd day	75		16.0
3rd day	73	0.5	
4th day	80		
5th day	84	0.1	

Figure 2 shows the changes in the erythrocyte counts of 2 human subjects, which were produced by the ingestion of one-quarter pound of butter and 400

mgm. of choline chloride in addition to a regular breakfast taken at zero time (fig. 2). Although the erythrocyte numbers fluctuated considerably, it will be seen (fig. 2) that they were reduced below normal much of the time during the first 36 hours, and had returned approximately to normal at 48 hours following the fat and choline meal. Mild reticulocytosis was observed from the 3rd to the 5th day following the experimental meal. Normal values of 0.3 and 0.4 per cent reticulocytes increased to 1.4 and 3.0 per cent respectively.

Discussion. The rapid lowering of red cell counts (fig. 1) and the increased icteric indices (table 1) that resulted from the administration of fat and choline indicate that the anemia is due to hemolysis of erythrocytes. Previous work (1) indicated that early discontinuation of fat feeding allowed the cell counts and icterus indices to return to normal. Other work (5) showed that the feeding of choline alone did not lower the erythrocyte counts in dogs until it had been administered daily for 8 or more days. Yet the simultaneous administration of choline was found to be necessary for fat feeding to lower the red cell count (1). Our conception of the mechanism by which choline and fat produce anemia has been stated previously (1). We believe that the products of fat digestion which have escaped resynthesis to neutral fat during absorption probably cause the destruction of red blood cells in these experiments, while the choline (by increasing the blood and oxygen supply to marrow) serves as a weak "brake" in preventing extra or compensatory activity of the bone marrow.

A comparison of the results reported here (fig. 1) with our previous results (1) seems to indicate that 2 doses daily of fat and choline cause a somewhat greater amount of hemolysis than one dose, daily, of each.

The depression of erythrocyte counts observed in 2 human subjects following a single fat and choline feeding (fig. 2) indicates that cells were hemolysed in this experiment, especially in view of the delayed reticulocytosis which followed in subsequent days.

CONCLUSIONS

The administration of 2 doses each, daily, of 60 grams of fat and 10 mgm. per kgm. of choline chloride to 3 normal dogs caused rapid reductions of 20 to 37 per cent in their erythrocyte counts, accompanied by significant elevations of icteric indices.

The addition of one quarter pound of butter and 400 mgm. of choline to the breakfasts of 2 human subjects caused depression of their red blood cell counts during the following 36 hours, succeeded by a subsequent reticulocytosis.

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THE EFFECT OF CERTAIN SUBSTANCES ON CLOTTING TIME, IN VITRO[1]

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Various investigators have added known chemical compounds to clotting mixtures of thrombin and plasma or whole blood, or to solutions of purified fibrinogen in attempts to elucidate the coagulation mechanism. Mueller and Sturgis (1) reported that cysteine hydrochloride would prevent the coagulation of whole blood under anaerobic conditions. The same inhibitory effect was reported by Sterner and Medes (2) for cysteine, taurine, and taurocholic acid. Several compounds containing thiol groups (cysteine, glutathione, ergothioneine, and thiolhistidine) were found by Kühnau and Morgenstern (3) to inhibit coagulation of human and porpoise blood. The most pronounced inhibition was exerted by the sulfhydryl form of glutathione (A-effect). If the SH group was blocked, however, the inhibition was replaced by a slight acceleration of clotting (B-effect). *In vitro* acceleration of the coagulation of whole blood in the presence of nicotinic acid was claimed by Calder and Kerby (4). This finding was refuted by Aggeler and Lucia (5) who substituted plasma for whole blood and observed a delay in clotting time. Chargaff and Bendich (6) produced coagulation of purified fibrinogen solutions with a number of synthetic agents in the absence of thrombin. They, too, were able to inhibit this effect by the addition of reducing substances like cysteine and sodium bisulfite. The only clear-cut evidence of acceleration of coagulation by chemical substances was suggested by Seegers and Smith (7) when gum acacia was present in clotting mixtures. Seegers and Smith ascribed this phenomenon to the increased sensitivity conferred on fibrinogen by the presence of additional colloid. Disagreement with this colloidal explanation will be discussed.

Preliminary observations indicated that gum acacia and phenol, alone or in combination, when added to thrombin solutions decreased the time required for the thrombin to coagulate oxalated beef plasma. The degree of potentiation was striking, amounting in some instances to an apparent ten-fold increase of activity when computed in terms of thrombin units according to the method of Jacques (8). It seemed of interest to determine whether other compounds under comparable conditions would exert a similar effect. The list of compounds tested is admittedly not exhaustive but an attempt was made to select a wide range of common chemicals. Altogether, 35 compounds were tested for their effect on the coagulation process. Several were found to give similar potentia-

[1] From data taken from the thesis submitted by C. F. Gerber in partial fulfillment of the requirements for the degree of Master of Science at New York University, Washington Square, New York. This author wishes to thank Dr. M. J. Kopac of New York University for his continued interest throughout the course of this work.

tion. Some compounds had no effect whatever. Certain other compounds were found to be distinctly inhibitory.

EXPERIMENTAL. *Procedure.* The procedure for measuring clotting time is based on the method for quantitative assay of thrombin and prothrombin suggested by Jacques (8). Minor modifications have been adopted for convenience. Because of its more ready availability, oxalated beef plasma, aged by storing for at least one month in the ice chest, has been substituted for aged horse plasma. The clotting mixtures used in this laboratory contained 0.1 cc. of aged oxalated beef plasma, 0.1 cc. of diluted imidazole buffer, and 0.4 cc. of thrombin solution. These mixtures were found to be preferable to Jacques' mixtures of 0.3 cc. of plasma, 0.1 cc. of buffer, and 0.2 cc. of thrombin solution. Greater sensitivity was obtained by the larger thrombin/plasma ratio without sacrificing the linear relationship between the logarithm of clotting time and the logarithm of thrombin concentration.

Wherever possible the thrombin concentration of a solution was adjusted to give a clotting time falling within the interval between 15 and 45 seconds. This was found to be the most reliable range for Jacques' calibration curve. Solutions having shorter clotting times are subject to serious variation in absolute value because of the delay introduced upon starting the timer. Beyond the 45 second limit, end-points are frequently difficult to distinguish because the coagulum, instead of producing a sharp instantaneous transition, tends to become diffuse.

To establish the effect of a given compound on coagulation some arbitrary concentration was prepared in saline. The solution was adjusted to the range between pH 7.0 and pH 7.5, most frequently to pH 7.4. This solution was used as the menstruum for a weighed sample of dried thrombin. If coagulation was accelerated at the chosen concentration, several other concentrations were selected in order to obtain an approximate optimum. However, if coagulation was delayed at the chosen concentration, dilutions were made until clotting time reached or approached normal or until some dilution exhibited acceleration of coagulation. Some potentiators were observed to exercise a destructive effect which progressed with time of contact with thrombin. As a standard procedure, therefore, the thrombin was triturated with 0.2 cc. of saline, diluted to the required volume with the experimental menstruum, and tested as rapidly as possible. This procedure reduced to a minimum the destruction which frequently accompanied the potentiation.

A control value, the clotting time for thrombin in saline, was established for each experimental compound. This was necessary since the experiments were performed with different samples of thrombin of varying purity and with different samples of plasma of varying sensitivity. The optimum concentration for any compound is valid since all concentrations of that compound were tested in identical systems. On the other hand, the optima for the several potentiators are only roughly comparable with one another because of the unavoidable variations in conditions. The most significant factor is the variation in sensitivity of the plasma for test purposes. This condition was especially difficult to control because the plasma samples were derived from beef blood collected under commercial conditions at a local slaughter house.

Where activity is expressed in terms of units, the Iowa unit defined by Warner, Brinkhous, and Smith (9) is intended.

The potentiation effect. An intimation of the extent of the potentiation effect may be obtained by referring to table 1. The controls represent successive dilutions of the same thrombin sample previously standardized according to Warner, Brinkhous, and Smith (9). The clotting times for these solutions were used to establish a calibration curve (Jacques (8)). From this curve the clotting times of the experimental solutions in 2 per cent gum acacia were converted into their relative activities expressed in units/cc. of solution. The first two values in table 1 must be disregarded in computing the degree of potentiation because such short clotting times contain an inherent error as previously noted. For the thrombin sample used to compile the data in table 1 the degree of potentiation is 7.5 times. Other thrombin samples responded to the potentiators to varying extents. For any one preparation, however, there is a constant degree of potentiation regardless of the amount of thrombin present in the test solution.

TABLE 1

Comparative clotting times of varying concentrations of thrombin in saline and in the presence of 2 per cent gum acacia

CONCENTRATION OF THROMBIN IN BOTH CONTROL AND EXPERIMENTAL SOLUTIONS	CLOTTING TIME: Control—thrombin in saline	CLOTTING TIME: Experimental—thrombin in 2 per cent gum acacia	CALCULATED ACTIVITY, CORRESPONDING TO EXPERIMENTAL CLOTTING TIME	DEGREE OF POTENTIATION $\frac{\text{EXPERIMENTAL ACTIVITY}}{\text{CONTROL ACTIVITY}}$
(units/cc.)	*seconds*	*seconds*	*(units/cc.)*	
20	15	3	200	10 ×*
10	25	5.5	85	8.5 ×*
5	40	10	36	7 ×
2.5	65	15	20	8 ×
1		30	7.5	7.5 ×

* These values for potentiation are less valid because they are calculated from experimental clotting times falling outside of the reliable range of the calibration curve.

Thrombin is the substance affected. The test compounds, *per se,* are incapable of coagulating fibrinogen. This was demonstrated by using clotting mixtures which contained all the components except thrombin. None of the test compounds, with the exception of salicylaldehyde, coagulated the fibrinogen of the test plasma in the absence of thrombin. Salicylaldehyde produced coagulation only after a prolonged period of time. This observation is in contradiction with the findings of Chargaff and Bendich (6) who reported that purified fibrinogen solutions could be coagulated by the addition of chloramine-T, ninhydrin, alloxan, salicylaldehyde and other synthetic agents. The apparent discrepancy may well be due to the use by Chargaff and Bendich of purified fibrinogen solutions which have considerable colloidal instability (c.f. Jacques (8)). Ferguson and Ralph (10) found that ninhydrin mixed with fibrinogen produced a flocculent granular deposit that was in no sense a coagulum. They support this contention by dark-field examination.

The compounds under consideration have no effect upon prothrombin. With

a solution of gum acacia or phenol as the menstruum, no coagulation occurred when purified prothrombin prepared according to Astrup and Darling (11) was substituted for thrombin in the clotting mixture. Addition of a calcium salt in the absence of cephalin to the prothrombin-test compound mixture also failed to produce coagulation. There also was no coagulation when cephalin[2] alone was added to identical mixtures. Therefore, the potentiators have no determinable effect on prothrombin and, acting alone, cannot mediate the conversion to thrombin. Hence, the compounds cannot substitute for calcium or thromboplastic substances in the process of conversion.

The possibility remained that the observed phenomenon might be due to some contaminant present in the thrombin samples. To eliminate this possibility, samples of thrombin were tested which had been prepared from beef plasma and rabbit plasma by several different methods (11–13). A sample of human thrombin from the laboratory of Dr. E. J. Cohn[3] (Harvard) was also tested. Regardless of the species or the mode of preparation, the thrombin was affected in a qualitatively similar manner by the given compounds.

TABLE 2

Comparative clotting times in the presence of potentiating substances, given at their experimentally determined optima

Concentrations on either side of the optima give longer clotting times than the listed values.

COMPOUND	OPTIMUM CONCENTRATION	CLOTTING TIME	
		Experimental	Control
	per cent	*seconds*	*seconds*
Gum acacia	5	8	40
Phenol	0.2–0.5	18	30
Catechol	0.5	20	36
Resorcinol	0.1–0.3	27	36
Hydroquinone	0.25	31	36
Pyrogallol	0.2	26	36
Phloroglucinol	0.075	26	36
o-Aminophenol	0.3	18	36
p-Aminophenol	0.3	27	36
Salicylaldehyde	0.12	15	24
o-Cresol	0.75	5	25
m-Cresol	0.5	10	25
p-Cresol	0.5–0.75	7	25
Picric acid	0.1	17	26

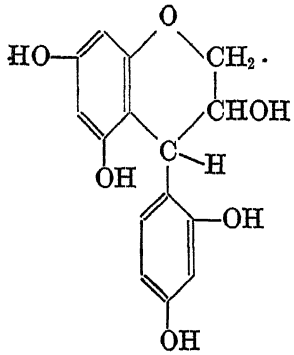
Effect of various chemical substances. Those compounds which accelerated coagulation under the experimental conditions are given in table 2 with their approximate optima as determined in the manner described. Higher or lower concentrations of the compounds give longer clotting times than the listed values.

[2] Kephalin, purchased from Armour Laboratories, Chicago, Illinois.

[3] Sample obtained through courtesy of Dr. M. J. Kopac, New York University.

Except for gum acacia, the potentiators are all phenolic compounds. This exception may bear closer relationship to the phenols than appears evident at first glance, as is suggested below.

Gum acacia is somewhat unique among the potentiators inasmuch as it is not a distinct chemical entity. Although principally carbohydrate, commercial gum acacia is a complex mixture, containing also some amino acids, tannins, oxidases and other enzymes, and traces of magnesium, potassium, and calcium. One of the contaminants reported (14) is the tannin, acacatechin, possessing the following structure:



While the possibility is just as strong that some other unknown constituent may be responsible for the potentiation, acacatechin must be considered on the basis of structural identity with the phenols.

Repeated attempts to isolate and characterize the active constituent of gum acacia have been unsuccessful. However, these experiments have demonstrated the stability of the active substance and have served to eliminate certain factors from consideration. For example, solutions of gum acacia have been kept at room temperature without any precautions for indefinite periods of time and still exhibited no diminution of potentiating activity. In fact, contamination by heavy mold growth in one solution did not reduce the effect. The active principle is not extractable with hot solvents. It resisted destruction when boiled in saline for one hour and was also unaffected when subjected to 24-hour digestion with pepsin at 37°C. These last two treatments destroyed the oxidase which contaminates gum acacia. This was indicated by failure to produce a blue color when benzidine was added in the presence of hydrogen peroxide (15).

Seegers and Smith (7) attributed the observed decrease in clotting time to the colloidal properties of gum acacia. Objection to their colloidal explanation is substantiated by the following experiment: A 10 per cent solution of gum acacia in constant boiling HCl (20 per cent) was heated under reflux for two hours. The major portion of the carbohydrate was carbonized by this treatment. The carbonaceous material was filtered off, the filtrate neutralized and dialyzed free of salt. The solid content of the dialyzed filtrate was less than 0.05 per cent, representing less than 0.5 per cent of the original gum acacia. In spite of this treatment, the solution proved capable of accelerating clotting

time, although the effect was diminished appreciably. Obviously the colloidal nature of gum acacia is destroyed and hence can no longer be considered a factor in the observed phenomenon. Confirmation for this point of view is given by the inclusion of pectin and gum tragacanth in table 4. Although these substances provide additional colloid substrate, they are inhibitors and not potentiators or stabilizers.

Those compounds which exerted no effect on the coagulation process are listed in table 3. The given concentrations are the highest which were tested. Two of these, phenylcellosolve and p-hydroxybenzoic acid, are phenolic derivatives. In both instances, substitution has destroyed the phenolic nature of the molecule. The introduction of the highly oxidized carboxyl group into p-hydroxybenzoic acid tends to produce a stable molecule. Phenylcellosolve possesses a free hydroxyl group which is alcoholic rather than phenolic. The inactivity of these compounds indicates that readily reactive phenolic groupings are essential to the potentiation phenomenon.

The inhibitory substances have been set down in table 4. The given concentrations are the dilutions at which the inhibition tended to disappear. In some

TABLE 3

Substances which have no effect on clotting time

COMPOUND	CONCENTRATION	CLOTTING TIME	
		Experimental	Control
	per cent	*seconds*	*seconds*
p-Hydroxybenzoic acid	0.3	37	36
Phenylcellosolve	0.3	25	25
Riboflavin	(sat'd) 0.12	25	25

instances the concentrations are the lowest which were tested. Higher concentrations of the substances give prolonged clotting times. In some instances coagulation is completely prevented at high concentrations.

DISCUSSION. The term "potentiation" is applied to the observed phenomenon because of the apparent increase in thrombin potential conferred by the presence of the accelerating compounds in the clotting system. Probably the net effect of thrombin upon fibrinogen is not altered by the addition of these substances, but their presence in the system enables thrombin to perform its normal function more rapidly. Examination of the data may afford some explanation for the mode of action of the potentiators and for the normal interaction between thrombin and fibrinogen.

It was pointed out in connection with table 2 that, except for gum acacia, all of the potentiators are phenolic compounds. The inactivity of p-hydroxybenzoic acid and phenylcellosolve (table 3) indicates that readily reactive phenolic groupings are necessary for potentiation. Tyrosine and epinephrine, naturally occurring phenols, are listed in table 4 as inhibitors. Both compounds are readily oxidizable phenols, but the oxidation is irreversible. According to Ball

and Chen (16), epinephrine is probably oxidized to an orthoquinone in the ordinary manner. However, this quinone is destroyed with such extreme rapidity that it is impossible to form an oxidation-reduction system with epinephrine. In a like manner, according to Raper (17), tyrosine is oxidized to o-dihydroxyphenylalanine and then to the ortho-quinone. Reversibility of the reaction is prevented by internal rearrangement and ring closure. Inhibition by these natural phenols indicates that the potentiation phenomenon requires not only reactive phenolic groupings but also that the phenolic groupings be reversibly oxidizable.

TABLE 4

Comparative clotting times in the presence of inhibitors, given at the concentrations at which the inhibition tended to disappear

Increases in concentration give prolonged clotting times.

COMPOUND	CONCENTRATION	CLOTTING TIME	
		Experimental	Control
	per cent	*seconds*	*seconds*
Pectin	0.01	57	36
Gum tragacanth	0.5	49	38
Sodium bisulfite	0.05	51	36
Cysteine	0.0001	40	36
Cystine	0.01	52	40
Glutathione	0.01	28	25
Potato oxidase*	(dil'd 10×)	54	40
Chloramine-T	0.0035	31	17
Ninhydrin	0.08	no clot	17
o-Phenylenediamine	0.00001	24	24
p-Phenylenediamine	0.0001	24	24
l-Ascorbic acid	0.0001	24	25
d-Isoascorbic acid	0.01	26	25
d-Glucoascorbic acid	0.01	25	25
Synkayvite "Roche"†	0.015	30	25
Nicotinic acid	0.05	34	25
l-Tyrosine	0.01	26	26
l-Epinephrine	0.1	48	26

* Crude potato press juice.

† Tetra-sodium salt of 2-methyl-1,4-naphthohydroquinone diphosphoric ester.

These facts suggest that an oxidation-reduction mechanism is involved. The concept of an oxidation-reduction mechanism of blood coagulation is not new. In speculating upon the manner of coagulation of fibrinogen by their experimental agents, Chargaff and Bendich (6) proposed that coagulation resulted from the oxidation of some susceptible grouping of the fibrinogen molecule. From their experimental findings, Chargaff and Bendich suggested that aminoacyl groupings were the most likely points of attack. Baumberger (18), on the basis of photodynamic coagulation of fibrinogen, postulated that coagulation occurred as a consequence of the oxidation of sulfhydryl groups. Baumberger concluded

that clotting can occur only when the thiol groups of fibrinogen are in the reduced or sulfhydryl form and that thrombin is active only in the oxidized or disulfide form. In Baumberger's theory the mechanism of coagulation involves oxidation of fibrinogen sulfhydryl linkages to the disulfide form with concomitant reduction of thrombin disulfide linkages to the sulfhydryl form.

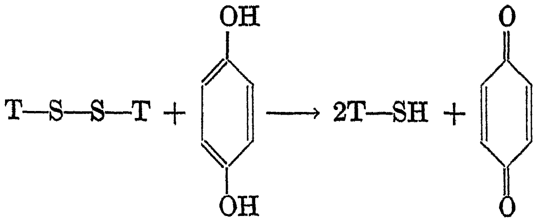
If active disulfide thrombin be represented as T—S—S—T and coagulable fibrinogen as F—SH, then the normal process of coagulation may be represented as follows:

$$\text{(i)} \qquad \text{T—S—S—T} + 2\text{F—SH} \rightarrow 2\ \text{T—SH} + \text{F—S—S—F}$$

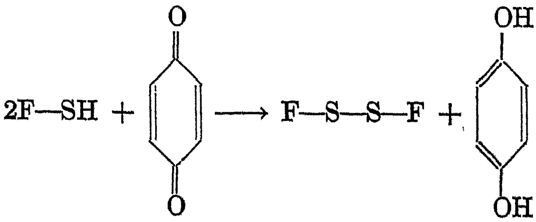
Fibrin is represented here as the disulfide form of fibrinogen (F—S—S—F).

Reversibly oxidizable phenols may well fit into an oxidation-reduction system. Because of the ease with which phenols are oxidized, they are excellent reducing substances. If a diphenol is added to the coagulating mixture, the formulation can be modified to read:

(ii)



(iii)



The net effect of equations (ii) and (iii) is identical with normal coagulation as expressed in equation (i); i.e., fibrinogen hydrogen is transferred to thrombin. The only difference lies in the rate of reaction as determined experimentally. The action of thrombin is thus potentiated because the phenols assume the rôle of hydrogen carriers mediating the normal oxidation of fibrinogen by thrombin.

A number of compounds in table 2 are monophenols. According to Raper (17) the first stage in the oxidation of a monophenol is the introduction of a second hydroxyl group in the ortho position. The oxidation then proceeds to the orthoquinone. Such a molecular modification would permit monophenols to play a rôle identical with that of the diphenols in mediating the clotting process.

The inhibitory substances listed in table 4 are of such diverse chemical nature that any unified concept of their action seems impossible. Because of the irreversible nature of the oxidation of tyrosine and epinephrine, clotting time would be prolonged since only the reduction of thrombin, equation (ii), can occur. The action of such active reducing substances as sodium bisulfite, cysteine, and

glutathione is probably similar. The reduction of thrombin probably takes place but the oxidative cycle, involving hydrogen transfer to fibrinogen, is not completed resulting in prolonged clotting time. A similar inhibition was reported for sulfur compounds by Kühnau and Morgenstern (3). The slight acceleration of coagulation when the SH group of glutathione was blocked indicated that the inhibition reported by Kühnau and Morgenstern depended on the active reducing properties of the compounds. The ascorbic acids, also, probably belong in this category. They are easily oxidized but do not seem able to mediate the necessary hydrogen transfer.

Chloramine-T and ninhydrin are good oxidizing agents. Their ability to interfere with coagulation would appear to stem from their ability to maintain thrombin in the disulfide form. Ferguson and Ralph (10) also observed a prolongation of clotting time when thrombin in ninhydrin solution was added to purified fibrinogen.

On the basis of oxidation-reduction behaviour analogous to the phenols, the phenylenediamines might be expected to exhibit a reasonable degree of potentiation. A possible explanation for the failure to demonstrate potentiation might be found in the destructive effect on thrombin exhibited by some of the potentiators. If the rate of destruction markedly exceeded the rate of potentiation, then the substances would appear to be inhibitory.

The authors are indebted to Mr. Charles Anderson of this laboratory for assistance in preparing thrombin samples.

SUMMARY

1. The coagulation of fibrinogen by thrombin can be accelerated by the addition to the coagulation mixture of various phenolic substances and gum acacia. The addition of other common reducing substances prolongs clotting time.

2. Blood coagulation appears to be a process involving oxidation-reduction, operating possibly through the oxidation of susceptible sulfhydryl groupings of fibrinogen to produce fibrin.

3. Potentiation of the thrombin-fibrinogen interaction by phenolic substances affords confirmatory evidence for the probable oxidation of fibrinogen by thrombin.

4. A phenolic substance, in order to exhibit potentiation, must possess free, oxidizable hydroxyl groups and the oxidation must be reversible.

5. The hypothesis is offered that the reversible oxidation-reduction of the active phenols permits them to accelerate the oxidation of fibrinogen by thrombin, with the phenols assuming the rôle of hydrogen carriers.

6. It is suggested that reducing agents other than phenols, although able to reduce thrombin to the inactive sulfhydryl form, are inhibitory because they are unable to complete the cycle by accepting hydrogen from fibrinogen.

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OSMOTIC AND ELECTROLYTE CONCENTRATION RELATIONSHIPS DURING THE ABSORPTION OF AUTOGENOUS SERUM FROM ILEAL SEGMENTS[1]

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The fact that water and solutes are rapidly absorbed from autogenous serum placed in intestinal segments (1, 2) is strongly suggestive evidence that normal osmosis is not the major driving force in water movement from gut to blood. Recent studies with isotopic tracers (3) have given more convincing evidence for this conclusion. The studies reported here were designed to elucidate further the phenomena associated with absorption of water from physiologically isotonic solutions. Autogenous serum placed in the gut provides a situation in which initially no important concentration difference exists between the intestinal lumen and the blood vessels in the intestinal wall. Therefore initially normal osmosis cannot cause net transport of water. Only two types of processes could conceivably alter this state of concentration equilibrium: (a) active transport of materials into or out of the gut by the intervention of the intestinal epithelial cells, or (b) changes in the materials of the serum within the lumen of the gut, as by enzymatic digestion. All known types of intestinal digestive enzymes are hydrolytic and by their action would tend to increase the number of osmotically active particles in solution. Thus such an action should result in an increase in osmotic activity, the reverse of what will be shown to occur, and should furthermore cause a net flow of water by osmosis into the gut, also the opposite of what is known to happen. One is left with the first type of process to account for the actually occurring changes.

METHOD. Dogs which were de-wormed three weeks or more previously with two weekly doses of 2.5 cc. of tetrachlorethylene were anesthetized with nembutal, 30 mgm. per kgm. of body weight, intraperitoneally. Carefully washed ileal loops were prepared as described previously (3). Quantities up to 100 cc. of the animals' blood were removed and allowed to clot in a centrifuge tube. Measured quantities, between 10 and 50 cc. of the serum, were placed in ileal loops and samples removed at twenty-minute intervals. These samples, and arterial blood specimens taken at the beginning and end of the period of observation, were subjected to study. Chloride, carbon dioxide and sodium were determined by the techniques previously employed (4). The pH was measured with the Leeds and Northrup glass electrode. The total osmotic activity was measured by the Hill-Blades (5) thermoelectric vapor tension technique.[2] Special attention was paid to the carbon dioxide tension in the thermocouple chamber in the

[1] Aided by grants from the Rockefeller Foundation and the Graduate School of the University of Minnesota.

[2] Osmotic activity is expressed in terms of the concentration of sodium chloride in millimols per kilogram of water having the same vapour tension.

osmotic activity measurements. In two cases the carbon dioxide tension in an artificially created gas phase in the intestine was measured and the equilibrating gas mixture made up to that concentration. In one instance such an intestinal gas phase was withdrawn and used to fill the thermocouple chamber of the Hill-Baldes apparatus. These controls showed that 5 per cent carbon dioxide in the chamber gave reliable results. All readings were made immediately after arrival at temperature equilibrium in the chamber. These precautions were taken in order to make certain that artefacts due to carbon dioxide loss or microbial or enzymatic action did not vitiate the results. Possible composition changes before

TABLE 1

Chemical composition and osmotic activity changes during absorption of autogenous serum from ileal segments

EXP. NO.	GUT SEGMENT CONTENTS												SERUM	Δ O.A. in 40 min.	Δ[Cl] mE/min.
	Chloride mE/L		Carbon dioxide mM/L		Sodium mE/L		pH		Volume change per cent	Osmotic activity equivalents of NaCl mM/kgm. H_2O			Osmotic activity		
	0 time	40 min.	0 time	40 min.	0 time	40 min.	0 time	40 min.		0 time	20 min.	40 min.	Average		
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)
1	110	80	22	35					−49	156.9	147.0	133.5	156.9	−23.4	−0.8
2	99	70	28	51	152	140			−51	155.0		143.5	155.3	−12.5	−0.7
3	109	76	32	47	153	140	7.51	7.44	−51	156.5	149.4	139.6	155.2	−15.6	−0.8
4	109	74	32	58			7.30	7.35	+52	164.0	161.3	159.6	167.0	−7.4	−0.9
5	109	68	22	45	162	162			−50	159.0		149.1	160.1	−11.0	−1.0
6	103	85	29	41	149	143	7.39	7.39	−62	164.0		151.0	157.5	−6.5	−0.6
7	112	82	27	47	156	133	7.38	7.42	−60	164.0		147.5	157.5	−10.0	−0.8
8	123	63	25	55					−65	156.0	143.5	142.5	153.2	−10.7	−1.6
9	112	69							−57	156.5	147.2	138.5	156.5	−18.0	−1.2
10*	112	106							+5	156.5	154.5	152.8	156.5	−3.7	−0.4
11†	111	106	29	33	148	148	7.13	7.17	+256	162.5		159.5	160.8	−1.3	−0.1
Mean‡	110 (107)	74 (76)	27 (28)	47 (46)	154	144	7.40	7.40	−44	159.1	149.7	144.9	157.7	−12.8 (−11.1)	−0.9

* 0.003M $HgCl_2$ added.
† 0.004M $HgCl_2$ added.
‡ Excluding experiments 10 and 11. The numbers in parentheses are means for experiments 2, 3, 5, 6 and 7.

drop deposition on the thermocouple were minimized by storage at icebox temperature when immediate analysis was not made.

Results and discussion. The major observations in eleven experiments are presented in table 1. It can be seen in column 9 that in all but one instance when unpoisoned serum was placed in an ileal loop there was a rapid absorption of water, amounting to more than 40 per cent of the volume in an hour. This is in confirmation of earlier observations (2). There is seen in columns 1 and 2 a regular and significant decline in chloride concentration. This decline, it may be noted, is in each case from the blood serum chloride level which is the original value. At the same time there is invariably an increase in carbon dioxide content as indicated in columns 3 and 4. The mean decrease in chloride is 36 mE/L, and the mean increase in carbon dioxide is considerably less, being 20 mM/L.

The ordinary change in sodium concentration is a decrease, the mean being 10 mE/L. The balance of ions is thus fairly well accounted for, since the chloride loss should equal the sodium loss plus the carbon dioxide gain, if the latter is present as bicarbonate or some electrochemical equivalent. One can use either the mean of all experiments or that of the experiments in which all three values were measured. In the later experiments the mean decline of chloride was 31 mE, of sodium 10 mE, and the rise in carbon dioxide 18 mE. The ion balance is thus accounted for within 3 mE/L. It is interesting to note, however, that there is a net decrease of inorganic electrolyte amounting to 12 or 13 mM/L depending upon which series is used. Unless there are compensating factors, that is, increases in concentration of other constituents, one would anticipate an approximately equivalent decline in osmotic activity.

It is therefore significant to note in column 14 that the mean difference in osmotic activity between the blood serum and the gut fluid (autogenous serum undergoing absorption) after the same period of time is 13 mM/kgm. H_2O, depending upon whether the total series or only the complete experiments are employed. Thus within the limits of error of measurement all of the osmotic activity change can be accounted for on the basis of NaCl movement from gut to blood against concentration gradients.

The fact that the osmotic activity of autogenous serum in ileal segments invariably declines with time, while water is being absorbed, throws light on the absorption phenomenon. Any hydrolytic reactions occurring would tend to increase osmotic activity, consequently it is believed that they play a negligible rôle in the changes observed. In two experiments low concentrations of mercuric chloride were added to the serum before introduction into the gut. In these, experiments 10 and 11, there was a much smaller change in osmotic activity, chloride and carbon dioxide, and no change in sodium concentration. This effect of mercuric chloride has been studied more fully in other experiments (6). It may be noted that higher concentrations are necessary in the presence of protein to produce poisoning than are required in NaCl or Na_2SO_4 solutions. Even 0.003 M $HgCl_2$ does not poison the intestine completely in the presence of serum, whereas 0.001 M produces large effects in inorganic salt solutions (7).

A few observations on the carbon dioxide tension and the hydrogen ion concentration were made on gut fluids. Since the total carbon dioxide content of the fluids was also known, the Henderson-Hasselbach equation could be applied to ascertain whether the autogenous serum in the gut for an hour behaved as normal serum does. In experiment 6, for example, the carbon dioxide tension in the gas phase over the fluid in the gut was 7.0 per cent of an atmosphere. The total carbon dioxide content of the fluid was 41 mM per liter. Using the value $pK^1 = 6.10$, one finds that the predicted pH value is 7.52, in place of the observed 7.39. Discrepancies in the same direction were seen in other experiments. This observation could result from a greater than normal content of some nonbicarbonate acid-labile carbon dioxide in the gut fluid. The form in which it may be is unknown but at any rate it is obvious that the value $pK^1 = 6.10$ cannot be applied directly to this system.

It may be noted in column 15, table 1, that in every instance when unpoisoned serum was placed in an ileal loop the rate of chloride concentration decline was 0.6 mE per minute or greater, whereas with $HgCl_2$ additions the rates were 0.4 mE per minute or less.

The fact that chloride impoverishment occurs during the absorption of autogenous serum deserves comment. Ordinarily this occurs only in the presence of a polyvalent anion (8). It does not occur within the first hour during the absorption of an isotonic sodium chloride solution, in which case the chloride concentration tends to approach the blood level (9). The results with serum suggest that the presence of protein alters the situation considerably. The iso-electric point of serum albumen is below the pH values encountered (column 7) and therefore the albumen is present as an anion. Since the protein cannot pass across the intestinal epithelium in significant amounts it would behave in this respect like polyvalent anions.

The findings noted above concerning a slight imbalance in ion shifts indicate that a somewhat larger portion of the sodium in the serum may be electrostatically neutralized by protein or some other unknown anion after forty minutes in the ileal segment than before. However more than eighty-five per cent of the sodium released by the loss of chloride can be accounted for by the carbon dioxide increase. Nevertheless it must be noted that the carbon dioxide need not be present entirely as bicarbonate. Indeed the calculations noted above concerning the pK^1 in the Henderson-Hasselbach equation applied to these data indicate that it is not. A significant fraction of the carbon dioxide must be present in some other form. It is impossible to say in what form it may be but it does not seem inappropriate to mention that with partial protein hydrolysis one would expect an increase in free amino groups, which could increase the fraction of carbamino carbon dioxide at any particular carbon dioxide tension and pH. It is of interest that the time for evolution of carbon dioxide from these "gut serum" samples was noted to be extraordinarily long as compared with normal serum under the same conditions of analytical technique in the Van Slyke apparatus. Thus another reason exists for believing that a significant fraction of the carbon dioxide is not present as bicarbonate ion.

However the carbon dioxide apparently is present in such form that its compound acts as a univalent anion both electrochemically and osmotically. The evidence on the electrochemical score has been given. The evidence as to osmotic activity appears in figure 1. Here it is apparent that within a fairly small margin the observed osmotic activity of "gut serum" samples agrees with predicted values obtained from the sums of sodium, chloride, and carbon dioxide concentrations, divided by two in order to permit comparison with sodium chloride as the reference solution. In this calculation an arbitrary correction is made for solid content, taken to be 9 per cent, in the serum. All observed values fall within 7.0 mM per kgm. H_2O of the predicted quantities by this calculation. Since carbon dioxide may constitute as much as one-third of the total "anion" in this calculation the observed agreement strongly suggests that the carbon dioxide compound exerts an osmotic activity equal to that of an

equivalent amount of bicarbonate. This could occur in a hydrolyzing protein system since the uncovering of each amino group would simultaneously free a carboxyl. The former could bind a molecule of carbon dioxide and the latter satisfy the valence of a sodium ion.

The experiments herein described appear to be important primarily because their results prove that with no initial concentration differences between intestinal lumen and blood there is nevertheless an active movement of materials from gut to blood. At the outset there are no concentration gradients and the

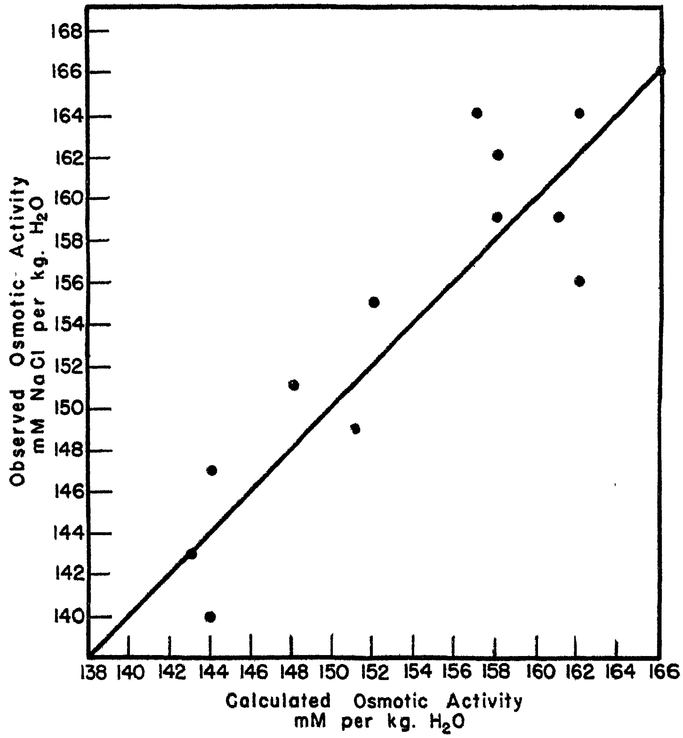

Fig. 1. A comparison of the observed osmotic activity of autogenous serum undergoing absorption from ileal segments with the values calculated from the sums of sodium chloride and carbon dioxide concentrations. Further explanations are given in the text.

subsequent development of concentration differences indicates an active participation of the membrane in the process. Net water movement between isotonic solutions cannot occur by normal osmosis, nor can the observed sodium and chloride transports occur by diffusion. Suitable inorganic salt solutions behave identically with serum in these regards (6). The serum experiments were performed primarily to obtain virtually complete identity of concentration of all materials on the two sides of the gut membrane, which is difficult to realize with artificial solutions. It should be noted that Heidenhain (2) measured the depression of the freezing point in serum undergoing absorption. He

found differences of 0.016 and 0.040°C. between the gut "serum" and the circulating blood serum. However, he did not control carbon dioxide loss and the results are therefore inconclusive. Nevertheless it is interesting that his observations agree rather closely with those reported here using improved techniques. His maximum hypotonicity was about 10 mM as against the average value of 13 mM obtained when the vapor tension method is used and precautions against carbon dioxide loss taken.

In the light of isotopic tracer studies (3) the data herein presented can be interpreted quite readily. It has been shown that there is a continuous movement of both water and uni-univalent electrolyte in both directions across the intestinal epithelium. The "apparent concentration" of univalent ions such as sodium and chloride in the water moved is much greater in the direction gut to blood than in the reverse direction. Thus the gut fluid would be expected to become impoverished in those ions, as it actually is. The impoverishment in electrolyte causes the observed hypotonicity, according to this reasoning. The isotope studies referred to above show further that the net rate of water absorption from hypotonic solutions is 200 times greater than could be accounted for on the basis of the water concentration gradient and simple osmosis. Thus one cannot accept the otherwise attractive hypothesis that the salt movement causes hypotonicity and that the latter might cause absorption by simple osmosis. Instead, although the ion impoverishment and hypotonicity are obviously interrelated, one is compelled to look further than to the hypotonicity to account for the water absorption. Somehow the osmotic pressure influences water absorption, but there are many possible ways in which this could occur without normal osmosis being the mechanism. For example, the osmotic pressure might influence the water content of surface epithelial cells and thus control their activities.

SUMMARY

1. Measurements have been made of the total osmotic activity, the volume, chloride, acid-labile carbon dioxide, sodium and hydrogen ion concentration changes in autogenous serum placed in ileal segments of anesthetized dogs.

2. There is rapid absorption of water and chloride from such serum. The sodium concentration falls less while the carbon dioxide content rises. The total osmotic activity falls. The average chloride concentration fall is 36 mE/L in 40 minutes. The average sodium loss is 10 mE/L in the same time. The average total carbon dioxide increase is 20 mM/L. The average net loss of inorganic electrolyte is 13 mM/L, which is identical with the measured decline in total osmotic activity.

3. There is a net absorption of sodium and chloride from autogenous serum in ileal loops, in spite of the fact that initially there are no concentration gradients present, and also in spite of the fact that with time the concentration in the gut falls much below that in the circulating blood.

4. The developing hypotonicity of originally isotonic solutions in ileal segments was predicted from isotopic tracer studies of absolute rates of movement of water and ions between gut and lumen and blood.

5. Reasons are presented for concluding that active processes occurring at or in the intestinal epithelium must be postulated to occur in order to account for absorption of salt and water from autogenous serum in ileal segments.

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THE PRODUCTION OF POLYCYTHEMIA BY COBALT IN RATS MADE ANEMIC BY A DIET LOW IN PROTEIN[1]

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Cobalt produces a marked polycythemia in the rat (Waltner and Waltner, 1929; Orten et al., 1931), the dog (Mascherpa, 1930; Davis, 1937), and in several other species of animals (Sutter, 1934). This effect does not occur, however, if the diet is lacking in iron or in copper (Orten, Underhill, Mugrage and Lewis, 1932), the latter observation confirming in a unique way the essential nature of copper for hematopoiesis. Previous work in this laboratory (Orten and Orten, 1943) has demonstrated and other recent work (Metcoff, Favour and Stare, 1945) has confirmed the fact that an adequate amount of dietary protein is essential for the maintenance of a normal concentration of hemoglobin in the blood of the rat. It therefore seemed possible that, if the organism is entirely dependent upon an adequate exogenous supply of protein for hemoglobin synthesis, cobalt administered to animals fed a diet deficient in protein should not produce a polycythemia as is true in normal rats. If, on the other hand, cobalt should produce a polycythemia under these experimental conditions, the importance of endogenous protein interchange in the organism would be reaffirmed in an entirely different and convincing manner.

The present study was designed to study these questions in the rat.

Experimental. A mild chronic anemia was produced in weanling female rats by the feeding of a diet low in protein (3.5 per cent lactalbumin) but adequate in all other respects, as has been previously described (Orten and Orten, 1943). After the anemia was well established by the feeding of the low-protein ration for a period of approximately 120 days, 10 of the rats were given cobalt orally, added to the daily vitamin supplements, in a dose of 0.5 mgm. cobalt (as recrystallized cobaltous sulfate) daily. The remaining 12 animals were continued on the unsupplemented low-protein diet as controls. A group of 10 normal rats fed the stock colony ration and given cobalt were also studied. The various groups of animals were observed for an 8-week period, hemoglobin determinations being made bi-weekly during that time on blood obtained from a tail vein. A photoelectric acid-hematin method was used. Body weights were determined weekly. Erythrocyte counts were made in duplicate on all animals at the end of the experiment by the usual method.

Results. The averaged hemoglobin values, together with actual minimum and maximum values recorded, are given in table 1.

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It is evident that a marked increase in the hemoglobin content of the blood occurred in the cobalt-treated normal rats as has been previously described. The values reached a maximum approximately 6 weeks after cobalt feeding was started. The mild chronic anemia persisted in the untreated, low-protein rats throughout the period of observation as has been reported elsewhere (Orten and Orten, 1943). The hemoglobin concentration of the blood of the cobalt-treated,

TABLE 1

Hemoglobin content of the blood of control rats and rats fed a low-protein diet supplemented by cobalt*

PERIOD	UNTREATED "LOW-PROTEIN" CONTROLS (12 RATS)	COBALT-TREATED, "LOW-PROTEIN" GROUP (10 RATS)	COBALT-TREATED NORMAL CONTROLS (10 RATS)
Initial	12.2 (10.3–13.5)	12.3 (11.5–14.5)	15.8 (13.7–16.9)
2-weeks	12.1 (10.9–14.2)	15.4 (13.9–17.8)	17.2 (16.3–18.9)
4-weeks	11.7 (10.2–14.2)	17.3 (14.5–19.3)	18.5 (15.9–21.6)
6-weeks	11.6 (10.5–13.3)	18.3 (16.3–20.0)	18.8 (16.4–20.7)
8-weeks	11.8 (10.3–13.6)	20.2 (17.8–22.0)	20.3 (16.6–24.2)

* Expressed as grams per 100 cc. blood. Group averages, with minimum and maximum individual values, are given.

TABLE 2

Erythrocyte counts of control rats and of rats fed a low-protein diet supplemented by cobalt*

GROUP	STOCK DIET	LOW-PROTEIN DIET
Untreated	8.2 (7.2–9.1)	8.1 (7.0–9.0)
Cobalt-fed	9.8 (8.1–11.3)	9.0 (7.6–10.8)

* Expressed as millions per cubic millimeters. Group average and individual minimum and maximum values are given.

low-protein anemic rats, however, showed a progressive increase at approximately the same rate as was found in the treated normal rats until typical high values were attained. Terminal erythrocyte counts confirmed the presence of a typical polycythemia in the cobalt-treated, low-protein animals. The average red cell count (table 2) found in these animals was increased over the control values as was that of the cobalt-treated normal rats. The degree of increase of the eryth-

throcyte values in the cobalt-fed, low-protein rats was somewhat more variable, however, than that which was found in the treated normal rats.

A very slight decrease in body weight occurred in six of the ten cobalt-fed, low-protein animals whereas a slight increase was found in the other four. There was no significant alteration in the average daily food intake. Likewise, there was little effect on the gross appearance of the animals, except for a reddening of the skin and eyes due to the development of the polycythemia.

Discussion. The foregoing results demonstrate that cobalt will produce a polycythemia in rats fed a diet low in protein as it does in animals given an adequate diet. The conclusion logically follows, therefore, that an adequate intake of dietary protein, although necessary for the maintenance of a normal content of hemoglobin in the blood under ordinary conditions, is not essential for hemoglobin synthesis in the face of a sufficiently powerful hematopoietic stimulus. Apparently, extra supplementary protein from the "metabolic pool" is either diverted from other uses to hemoglobin formation or additional body tissue protein is catabolized under the stimulus of cobalt administration and the degradation products are utilized for hemoglobin production. The results also confirm the previous findings that cobalt is a potent hematopoietic stimulant and suggest that the mechanism of the polycythemic action of cobalt, such as the production of local anoxia in the bone marrow (Orten, 1936) or the inhibition of respiratory enzyme systems (Barron and Barron, 1936), must be one effective in the anemic animal as well as in the normal.

The fact that there was no consistent loss in body weight nor gross evidence of abnormalities in the cobalt-treated, low-protein rats may be interpreted as indicating that the toxicity of cobalt is not greater in animals fed a diet low in protein than in adequately fed rats. This finding is of interest in connection with the observation (Griffith, Pavcek and Mulford, 1942) that methionine, cystine, and cysteine each decrease the toxicity of cobalt. It should be added, however, that the rats of the present study, although consuming a diet low in protein, are ingesting a protein, lactalbumin, which contains a relatively large amount of methionine, and also that the dosage of cobalt employed in producing polycythemia is considerably less than the toxic level.

CONCLUSION

The effect of cobalt on hematopoiesis in rats with a chronic anemia due to the feeding of a diet low in protein has been studied.

Cobalt produces a polycythemia in the low-protein animals at approximately the same rate and to about the same degree as in normal stock rats.

These results indicate that cobalt administration produces either a diversion of body tissue protein into hemoglobin synthesis or an increased utilization of the limited exogenous supply of protein for this purpose.

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OSMOTIC AND ELECTROLYTE CONCENTRATION RELATIONSHIPS DURING ABSORPTION OF SALT SOLUTIONS FROM ILEAL SEGMENTS

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In a preliminary report we (1) have described observations upon the osmotic activity of approximately isotonic salt solutions undergoing absorption in the ileum. This paper presents the results of sixty-two experiments in which osmotic activity and electrolyte concentration measurements have been made on isotonic solutions of salts and mixtures of salts, with and without the addition of small amounts of several poisons.

METHOD. Experiments were performed on 20 to 30 cm. ileal segments of nembutal anesthetized dogs prepared as has been described previously (2). The thermoelectric vapor tension method (3) had been employed for osmotic activity measurement Analyses for choline (4), sodium (5) and total carbon dioxide (6) were by the methods indicated.

RESULTS AND DISCUSSION. 1. *Sodium chloride-sodium sulfate solution absorption.* Observations on changes in osmotic activity of solutions, originally approximately isotonic, consisting of equi-osmotic fractions of sodium chloride and sodium sulfate are shown in table 1. These solutions were placed in ileal loops and small samples withdrawn at 20 minute intervals for 40 minutes or more. In column 1 are shown the values for the osmotic activity of the dog's plasma, taken under oil and kept from clotting with minimal amounts of heparin. Careful tests have shown that heparinized plasma and serum have identical osmotic activities under the conditions here employed. The mean value for plasma osmotic activity was equivalent to 161.5 mM NaCl per kgm. H_2O and the spread was between 157 and 166 mM. Inspection of the other tables shows the same range of values for plasma osmotic activity This normal spread of 10 mM in osmotic activity represents ± 3 per cent of the total, consequently it is impossible to prepare a solution which will, with certainty, be closer than that percentage to isotonicity for any particular dog. The osmotic pressure difference represented by 3 per cent of the isotonic concentration is about 150 mm. Hg, and is therefore by no means negligible in water transport studies.

The initial osmotic activities of the sodium chloride-sodium sulfate solutions employed varied on either side of the plasma value and the mean was 158.4 mM. Therefore the introduced solution was, on the average, 3 mM./l. hypotonic. Nevertheless, the osmotic activities after 20 minutes in the gut segment (column 3) were found in seventeen cases out of the twenty-one to have declined from the values at zero time, and in every case to be lower than the value for the dog's plasma. Likewise at 40 minutes the same situation prevailed. Thus in 81 per cent of cases the osmotic activity of the gut solution deviated down-

ward from the plasma value for 40 minutes. This is, of course, opposite to any prediction from considerations of normal osmosis since during this time water was being absorbed. In the other 19 per cent of cases the osmotic activity of the gut fluid remained hypotonic but it did not increase in hypotonicity relative to the plasma.

In column 5 are presented the differences in osmotic activity between the gut samples at 40 minutes and the blood plasma. The ΔO.A. always has a negative

TABLE 1

Osmotic activity changes during absorption of approximately isotonic solutions of $NaCl$-Na_2SO_4 mixtures from ileal segments

EXPT. NO.	OSMOTIC ACTIVITY mM NaCl PER KGM H_2O					RATE OF $[Cl^-]$ CHANGE
	Plasma ave. over period	Gut solution			Δ O.A.	Δ Cl
		Time 0	20 min.	40 min.		
	1	2	3	4	5	6
						mE./l./min.
1	160.5	144.3	149.4	154.2	−6.3	−1.6
2	162.7	157.4	156.0	157.0	−5.7	−1.8
3	166.2	159.9	160.1	157.3	−8.9	−1.2
4	164.6	156.2	153.8	152.3	−12.3	−1.4
5	164.6	166.6	158.7	156.3	−8.1	−1.7
6	162.5	160.0	155.5	153.5	−9.0	−1.4
7	158.9	159.5	157.5	156.5	−2.4	−1.2
8	161.6	159.5	157.2	159.8	−1.8	−1.7
9	161.0	160.0	154.5	152.8	−8.2	−1.6
10	161.0	160.0	151.6	156.0	−5.0	−2.1
11	160.6	159.8	156.0	155.0	−5.6	−1.1
12	160.6	159.0	152.6	153.0	−7.3	−1.2
13	163.0	159.9	157.5	157.1	−5.9	−1.1
14	163.0	158.2	154.4	157.2	−5.8	−1.7
15	160.8	156.8	157.0	156.8	−4.0	−0.6
16	160.8	155.4	157.8	156.2	−4.6	−0.9
17	160.6	156.1	154.4	153.7	−6.9	−1.3
18	160.6	155.6	154.4	155.9	−4.7	−1.4
19	159.6	160.3	154.4	153.4	−6.2	−1.7
20	160.3	160.4	156.1	158.6	−1.7	−1.9
21	157.4	160.4	156.4	155.4	−3.0	—
Mean.....	161.5	158.4	155.5	155.6	−5.9	−1.4

value, indicating gut fluid hypotonicity, and the mean is 5.9 mM. From these data it is apparent that the developing hypotonicity of such gut salt solutions during absorption is an entirely regular phenomenon.

In column 6 appear the data obtained by measurement of gut fluid chloride concentration at zero time and at 40 minutes. The initial values were all between 70 and 90 mE. It will be seen that the rate of chloride impoverishment was very high. A value of 2.0 mE. per liter per minute represents total

absorption within the 40 minute period from a solution containing 80 mE. per liter. The average decline was to 24 mE. per liter. As has been pointed out earlier (2) there is a concentration of sulfate associated with the chloride impoverishment.

2. *The influence of poison on sodium chloride-sodium sulfate solution absorption.* In table 2 appear the data from six experiments in which 0.001 to 0.003 M. $HgCl_2$ was added to approximately isotonic sodium chloride-sodium sulfate solutions placed in ileal segments. In sharp contrast to the situation in unpoisoned solutions it is to be seen from columns 1, 2, 3 and 4 that the gut osmotic activity tends to approach the plasma level, rather than to fall below it. The mean ΔO.A. is −0.7 mM., which is 12 per cent of the value seen in the absence of $HgCl_2$.

TABLE 2

Influence of $HgCl_2$ on osmotic activity changes during absorption of $NaCl$-Na_2SO_4 solutions from ileal segments

EXPT. NO.	POISON	OSMOTIC ACTIVITY mM NaCl PER KGM. H_2O					RATE OF [Cl^-] CHANGE
		Plasma ave. over period	Gut solution			Δ O.A.	
			Zero time	20 min.	40 min.		
		1	2	3	4	5	6
							mE./l./min.
1	0.001 M $HgCl_2$	158.3	150.2	156.1	158.6	+0.3	−0.2
2	0.001 M $HgCl_2$	163.5	150.1	158.3	161.5	−2.0	−0.1
3	0.001 M $HgCl_2$	158.8	160.4	158.9	158.2	−0.6	−0.1
4	0.001 M $HgCl_2$	159.5	160.4	159.0	158.8	−0.7	±0.0
5	0.003 M $HgCl_2$	158.8	160.4	159.8	159.0	+0.2	±0.0
6	0.003 M $HgCl_2$	159.5	160.4	160.2	158.2	−1.3	+0.1
Mean		159.7	157.0	158.7	159.1	−0.7	±0.0

Equally striking is the difference in chloride impoverishment between these experiments and the previous ones. Here the values, as seen in column 6, range between ±0.2, and the average is zero. There is no particular significance to the zero value, because it will be noted in experiment 6, one of two with 0.003 M $HgCl_2$, that the chloride concentration rose with time. The concentration of $HgCl_2$ determines the degree of poisoning. With higher levels the restriction to chloride movement into the gut is more completely lost (7). Poisoning with $HgCl_2$ produces at least two end effects, it prevents the occurrence of any significant hypotonicity (the mean value of −0.7 mM./kgm. H_2O for ΔO.A. is without statistical significance) and it prevents chloride impoverishment. Comparison with the results in section 6 will show that the failure in chloride impoverishment in the poisoned gut is associated with the absolute or relative increase in the rate of movement of chloride ion from blood to gut. These two end effects of poisoning may or may not be due to the same action.

3. *Sodium sulfate solution absorption.* The data from five experiments in which an isotonic or slightly hypotonic sodium sulfate solution was placed in ileal segments are shown in table 3. In these experiments in every case but one the gut osmotic activity tended to approach the plasma osmotic activity. The mean value for the difference between the gut osmotic activity at 40 minutes and the plasma value was found to be (column 5) −2.3 mM./kgm. H_2O. This value is significantly less than the −5.9 found in the case of isotonic sodium chloride-sodium sulfate solution mixtures. In only three out of twenty-one cases was the ΔO.A. in the latter series smaller than the highest value in the sodium sulfate series. It is concluded that the presence of an electrolyte, both ions of which are readily able to permeate the gut wall, is essential to the occurrence of hypotonicity in salt solutions undergoing absorption. Both sodium

TABLE 3

Osmotic activity changes during absorption of approximately isotonic solutions of Na_2SO_4 from ileal segments

EXPT. NO.	OSMOTIC ACTIVITY mM NaCl PER KGM. H_2O					RATE OF [Cl^-] CHANGE
	Plasma ave. over period	Gut solution			Δ O.A.	
		Zero time	20 min.	40 min.		
	1	2	3	4	5	6
						mE./l./min.
1	166.8	166.2	166.6	166.0	−0.8	+0.1
2	166.8	156.7	160.5	162.8	−4.0	+0.2
3	162.5	156.7	159.3	160.2	−2.3	+0.1
4	160.8	150.2	155.5	158.1	−2.7	±0.0
5	158.9	150.2	154.8	157.2	−1.7	+0.1
Mean.....	163.2	156.0	159.3	160.9	−2.3	+0.1

and chloride ions can so permeate the gut-blood barrier, but the sulfate ion is restrained, in common with all other non-toxic polyvalent ions which have been studied (8).

In column 6 of table 3 it will be noted that the change in the chloride concentration which was originally zero, was from ±0.0 to +0.2 mE./l. in the several experiments. The mean change was + 0.1 mE./l. That is to say that the chloride concentration in the gut fluid rose to 4.0 mE./l. over 40 minutes, which is to be compared with the plasma level of 100 mE./l. In one instance there was no measurable net entrance of chloride, and in one the value was double the mean. It is obvious that the gut epithelium allowed virtually no net transfer of chloride into the gut lumen. This is by no means equivalent to saying that chloride did not enter because there is every reason to believe that it did. However, if it did the rate at which it moved in the opposite direction (gut to blood) was in one case exactly as great, and in the other four, very nearly as great.

Observations on sodium movement using its radioactive isotope as a tracer

(9) bear upon this question. In those studies it was found that with approximately a zero concentration of sodium in the gut fluid, made to isotonicity with a magnesium salt, the absolute rate of sodium ion movement from blood to gut was about 0.3 mE./10 minutes, using loops containing 20 cc. of fluid. Thus the concentration change would have been 15.0 mE./l./10 minutes or 1.5 mE./l./minute if there had been no movement in the opposite direction. Unpublished observations in three experiments in which radioactive isotopes of sodium and chloride were employed simultaneously, have shown that under comparable concentration conditions the two ions move with approximately the same rates across the ileal epithelium. Therefore the figure 1.5 mE./l./minute is a value which can probably be transferred without serious error, to the case of chloride in the present experiments. Instead of this quantity we find 0.1 mE./l./minute. Obviously more than 93 per cent of the chloride ion entering the gut

TABLE 4

Influence of poisons on osmotic activity changes during absorption of Na_2SO_4 solutions from ileal segments

EXPT. NO.	POISON	OSMOTIC ACTIVITY mM NaCl PER KGM. H_2O					RATE OF $[Cl^-]$ CHANGE
		Plasma ave. over period	Gut solution			Δ O.A.	
			Zero time	20 min.	40 min.		
		1	2	3	4	5	6
							mE./l./min.
1	0.001 M $HgCl_2$	158.9	150.2	163.7	166.8	+7.9	+1.9
2	0.001 M $HgCl_2$	160.8	150.2	159.1	161.6	+0.8	+1.0
3	0.001 M $HgCl_2$	163.5	150.2	161.2	164.0	+0.5	+1.1
4	0.001 M $HgCl_2$	158.3	150.1	158.5	160.7	+2.4	+1.0
5	0.001 M Na arsenite	160.1	157.5	157.8	159.7	−0.4	+0.5
6	0.002 M Na arsenite	156.5	157.2	156.9	157.2	+0.7	+0.3
7	0.002 M Na arsenite	160.1	157.5	157.8	166.0	+5.9	+1.0
8	0.010 M Na arsenite	160.1	157.5	171.5	169.8	+9.7	+2.0
Mean		160.9	153.8	160.8	163.2	+3.4	+1.1

must have left it virtually simultaneously, according to this reasoning. Otherwise the chloride concentration should have risen to 60 mE./l. instead of to the observed 4 mE./l. in 40 minutes.

4. *The influence of poisons upon sodium sulfate solution absorption.* The results of eight experiments with slightly hypotonic sodium sulfate solutions in which $HgCl_2$ or $NaAsO_2$ in concentrations 0.001 to 0.002 M. were employed as poisons are presented in table 4. Here it is seen that in all but one instance the gut solution became hypertonic, the values in column 5 ranging from −0.4 to +9.7 mM./kgm. H_2O. This situation is the opposite of that seen with unpoisoned salt solutions, and is also in contrast with that found when isotonic sodium chloride-sodium sulfate solutions were poisoned. In the latter the end result was a virtually isotonic solution. With sodium sulfate solutions poisoned as indicated the increase in osmotic activity is associated with the

chloride movement. In column 6 it can be seen that there is a large influx of chloride ranging from 12 to 80 mE./l. concentration change in 40 minutes and averaging 44 mE./l. This finding could obviously account for the osmotic activity change if the chloride entered faster than the sulfate was lost. The sodium movement need not be concerned because substituting chloride for sulfate brings a 4 :3 increase in osmotically active ions.

The observed increase in osmotic activity with poisoned sodium sulfate could be accounted for qualitatively by assuming that a barrier to the entrance of chloride into the intestine had been broken down and that the entrance of chloride along its diffusion gradient, either exchanging with sulfate, or carrying a cation with it, caused the increase in osmotic concentration. However it is not certain that this simple explanation is correct. An alternative hypothesis is equally plausible, namely, that the poison alters the rates of active movement in the two directions across the intestinal epithelium. No attempt will be made at this time to decide between the alternatives.

5. *Volume change and osmotic activity.* A comprehensive picture of the relation between osmotic activity differences between the fluids in the intestine, and the blood plasma on the one hand, and the net water movement on the other, can be obtained by plotting the two variables against each other. This has been done in figure 1 for all of the experimental situations discussed in the previous sections. The most striking fact that appears is that with one exception which will be discussed, there was no instance of net water absorption (negative volume change) except when the osmotic activity of the gut fluid was lower than that of the plasma at 40 minutes after placing the fluid in the intestinal segment. In the one exception referred to the apparent absorption was 1 per cent, a value within the error of measurement, consequently the rule is apparently universal. This is in spite of the fact that, as pointed out above in table 1, many of the gut fluids were hypertonic at zero time. It is therefore a necessary condition for net water absorption that the intestinal epithelium must be able to decrease the osmotic activity of the fluid in the gut lumen. In no instance were solutions more than 3 mM./l. hypertonic employed in these studies, consequently it is not possible to be certain as to how far along the osmotic activity scale this action would appear.

On the other hand it is evident that such a rule in reverse does not prevail for intestinal volume increase. In nine out of twenty-three cases of volume increase the osmotic activity was less in the gut fluid than in the plasma. It is quite obvious that in these cases normal osmosis could not account for the volume increase. Perhaps secretion of the intestinal glands plays a rôle in this situation, but it is generally believed that these secretions are isotonic. Furthermore there is not a correlation between the magnitude of the volume increase and the extent of hypertonicity, as there should be if normal osmosis were responsible for the volume change.

Furthermore there is not a high correlation between the rate of volume decrease and the extent of hypotonicity. The points in the lower left quarter of the graph are very widely scattered, indicating that although developing hypo-

tonicity is invariably associated with water absorption, the osmotic force set up is probably not the driving force for water transport. If it were, the tendency should be for the points in the graph to be linearly related to the osmotic pressure gradient.

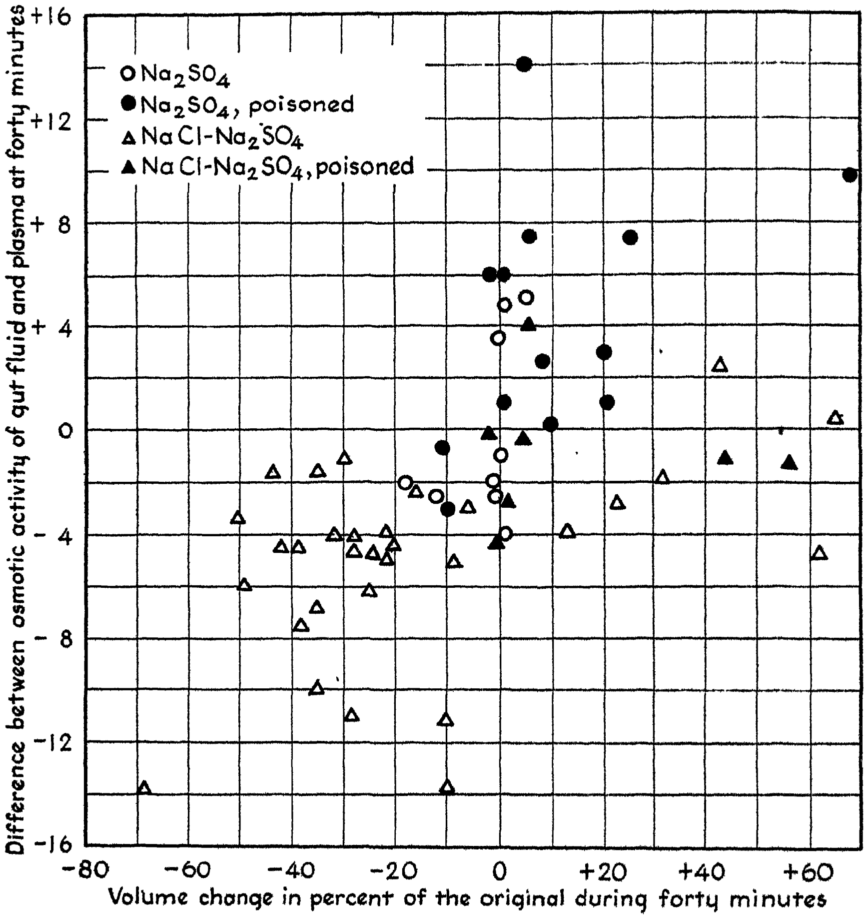

Fig. 1. Plot showing the relation between the water transport and the osmotic activity difference between gut segment fluids and the plasma, expressed in mM NaCl per kgm. H_2O, at forty minutes after insertion of the salt solutions indicated. The solutions were all originally approximately isotonic. In the case of the salt mixtures the two salts were present in equiosmotic proportions at the start.

The classical observations of Heidenhain (10) bear upon this problem. He reported that when significantly hypertonic sodium chloride solutions were placed in the ileum they tended to approach isotonicity, but that there was considerable water absorbed while the solutions were still hypertonic to the blood, as determined by the freezing point depression. The studies reported here confirm the conclusion of Heidenhain that forces other than those of normal osmosis determine the rate of water transport.

It should be noted that in every case in figure 1 in which water absorption occurred (negative volume change) to the extent of 20 per cent or more of the original volume in 40 minutes, the gut solution was the equiosmotic mixture of sodium chloride and sodium sulfate. When these solutions are absorbed the sodium chloride moves into the blood against a large concentration gradient. Very little or no sulfate ion is absorbed. Consequently the process causing hypotonicity is associated with the movement of uni-univalent salt against its concentration gradient, which requires the performance of work and is therefore an active process.

SUMMARY

Results of studies on osmotic and electrolyte concentration relationships during absorption of isotonic sodium sulfate and sodium chloride solutions from ileal segments in nembutal anesthetized dogs are reported. The influence of additions of 0.001 to 0.003 M $HgCl_2$ or $NaAsO_2$ was also studied. The osmotic activity was studied by the thermoelectric vapor tension technique. Special attention was paid to the carbon dioxide tension in the osmotic activity measurements.

When originally approximately isotonic solutions of sodium chloride and sodium sulfate in equiosmotic proportions are placed in ileal segments they invariably become hypotonic to the blood plasma. The average hypotonicity was found to be equivalent to 5.9 mM. NaCl/kgm. H_2O.

When 0.001 M $HgCl_2$ was added to such solutions the tendency to hypotonicity was virtually abolished. In the presence of this poison the impoverishment of chloride otherwise found was also abolished.

When originally isotonic sodium sulfate solutions were introduced into ileal segments such solutions were on the average 2.3 mM./kgm. H_2O hypotonic after forty minutes, a figure significantly less than that found when sodium chloride was present. The difference is believed to be related to the greater mobility of chloride than sulfate across the ileal epithelium.

The net increase in chloride concentration in sodium sulfate solutions was very small, on the average 4 mE./l. in forty minutes. Consideration of isotopic tracer data lead one to believe that this represents a small fraction of the chloride that entered in that time. The remainder must have returned to the blood by some active transport mechanism.

The addition of 0.001 to 0.002 M $HgCl_2$ or $NaAsO_2$ to isotonic sodium sulfate solutions in ileal segments caused those solutions to become significantly hypertonic, the mean value for osmotic activity being 3.4 mM./kgm. H_2O above the plasma. This was associated with a great influx of chloride, the average rate of increase in chloride concentration being eleven times that found in the unpoisoned solutions.

A study of the relation of net water movement and osmotic activity in the above mentioned experiments shows that in every case in solutions from which there was a significant water absorption there was definite hypotonicity of the gut solution as compared with the plasma at forty minutes after insertion of the fluid in the gut segment. It is concluded that when water is absorbed from

approximately isotonic solutions of sodium chloride and/or sodium sulfate, the intestinal mechanism is such that invariably the solutions become hypotonic within a short time. However there is not a significant positive correlation between the magnitude of the osmotic gradient between gut and blood and the rate of water absorption. It is believed that although a developing hypotonicity is a regular concomitant of water absorption from such solutions the osmotic gradient is not the driving force for water transfer.

When the net water transfer was zero, or in the direction of gut volume increase, the gut fluid osmotic activity was found to be sometimes lower and sometimes higher than that of the plasma. Entrance of water into the intestine occurred frequently when the fluid in it was hypotonic to the plasma, a result that could not occur from the action of normal osmotic forces. This observation constitutes further evidence that forces other than those of normal osmosis bring about water transport across the intestinal epithelium.

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RE-INNERVATION OF DENERVATED MUSCLE FIBERS BY ADJACENT FUNCTIONING MOTOR UNITS[1]

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The motor unit is usually considered as a functionally and anatomically independent structure, little or not at all affected by changes in the surrounding units. Thus it is tacitly assumed that if part of the innervation of a muscle is permanently destroyed, the remaining motor units representing the active part of the paretic muscle continue their normal function. No changes would occur in these active units, except perhaps some hypertrophy of their muscle fibers. However, observations of Mandelstamm (1882) and Exner (1884, 1885) cast some doubt on this simple concept. Exner found that the m. cricothyriodeus of the rabbit is innervated by the nn. laryngeus superior and medius. Severing of either of these nerves did not produce degeneration in the muscle, the transection of both gave the expected degeneration. Exner considered the possibility that muscle fibers which have lost their innervation by severing one of the nerves can obtain a new motor innervation from the other nerve before they have had time to degenerate. This hypothesis was supported by Lederer and Lemberger (1907) who concluded, on the basis of stimulation experiments, that Exner's results could not be explained by double innervation of each muscle fiber by both the nn. laryngeus superior and medius.

When we state Exner's supposition in the terms of the motor unit concept we would have to assume that after the removal of part of the innervation of a muscle, the remaining motor units grow by adopting muscle fibers which originally belonged to the denervated motor units. This possibility has been examined in the present investigation by a study of the force of contraction of muscles at various intervals after partial denervation.

METHODS. The muscles used were the mm. sartorius and quadriceps of the rabbit. The tendons of these muscles were dissected separately, leaving the muscle bellies covered as much as possible. Strings were tied to the tendons and the contractions were recorded isometrically under an initial tension of 50 grams in all instances.

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For stimulation an induction coil or a thyratron stimulator was used. Mammalian muscle can show a marked facilitation (Brown and Euler, 1938), so that the force of the contraction produced by single shocks depends to a considerable extent on the previous history of stimulation. This source of error can be eliminated by the use of short faradic stimuli for the determination of the force of contraction, and this method was used in all experiments reported here. Care was taken to stimulate with supramaximal stimuli. When using the inductorium, coil distances were chosen which caused a supramaximal break-, but no make shock. With comparable frequencies the force of contraction was the same using either of the two methods of stimulation in the same preparation.

Action potentials were recorded with a Mathews oscillograph, driven by a push-pull amplifier, and using silverchloride plated silver electrodes.

After the experiment the muscles examined were fixed in Zenker's solution, embedded, sectioned and stained with hematoxylin-eosine. In certain instances the surface of the cross section of muscle fibers was determined by drawing their outline with the help of a drawing prism on paper. About 100 of these outlines were cut out, weighed and compared with the weight of the outline of a known surface determined with the help of an object micrometer drawn at the same magnification. The weights of equal surfaces of different sheets of paper never varied more than a few per cent.

The innervation of the mm. sartorius and quadriceps. The m. sartorius of the rabbit is a thin and slender muscle situated superficially on the anterior and medial side of the thigh. The motor fibers for the sartorius muscle leave the femoral nerve with the n. saphenous, which gives off one or two thin motor nerves for this muscle.

The femoral nerve of the rabbit is formed for the greatest part by the 6th lumbar spinal nerve. Regularly a branch from the 5th lumbar spinal nerve joins the thick bundle from L 6 to form the n. femoralis. The femoral nerve gives off a thick branch connecting the 6th and 7th lumbar nerves which is also joined by a thin contribution of L 5. Usually there is present a thin connection between the 4th and 5th lumbar spinal nerves. The insert in figure 1 is a sketch of this part of the lumbar plexus. It should be understood that variations occur frequently and that especially the size of the connection between L 5 and L 6 varies considerably.

In 16 rabbits the contribution of the segments L 4, L 5 and L 6 to the motor innervation of the m. sartorius has been investigated by stimulating the respective spinal nerves or their relevant branches and by recording the force developed by the muscle. In these experiments the other muscles of the thigh had been denervated by severing the femoral nerve but for the n. saphenous, and by cutting the obturator and sciatic nerves. After recording the effects of stimulation of the spinal nerves, the femoral nerve was stimulated in the abdominal cavity to determine the total force developed by the m. sartorius. The rabbits used weighed 2.5 to 3 kgm. The results are collected in table 1. In several instances it was not possible to obtain reliable results from the stimulation of L 6 because of the shortness of this structure. It is obvious that there are

great variations in the participation of L 5 and L 6 in the innervation of the m. sartorius. In most animals the contribution of L 5 is small, much smaller than that of L 6, but in a few rabbits L 5 contributed most of the motor fibers for that muscle. In one animal (16) the stimulation of L 5 caused no contraction at all in the m. sartorius. There is a relation between the magnitude of the contribution of L 5 to the innervation of the muscle and the size of the connection between L 5 and L 6. The 4th lumbar segment as a rule does not participate in the innervation of the sartorius. In one animal (11), however, L 4 supplied

TABLE 1

Force in grams developed in the sartorius muscle by the faradic stimulation of L 4, L 5, L 6 and the femoral nerve

NO.	L 4	L 5	L 6	FEMORAL NERVE
1	0	68	77	136
2	0	29	105	127
3	0	11	—	181
4	0	81	55	128
5	0	17	—	102
6	0	57	123	162
7	0	21	160	176
8	0	52	69	107
9	0	32	—	122
10	0	69	74	148
11	8	179	35	194
12	0	10	98	95
13	0	36	—	164
14	0	13	—	211
15	0	41	154	191
16	0	0	—	110
Means	—	45	—	147
Standard errors	—	11	—	9

some motor fibers for this muscle. In this rabbit most of the motor fibers for the m. sartorius came from L 5.

It was found in some experiments in which the 7th lumbar spinal nerve, after being exposed from the dorsal side, was stimulated, that this segment does not supply any motor fibers for the m. sartorius.

The mean force produced by stimulation of the femoral nerve was 147 grams with a standard error of 9 grams. The mean force produced by stimulation of the fibers from L 5 is 45 grams with the relatively much greater standard error of 11 grams. This greater variability is explained by the fact that this figure includes the segmental variation in contrast to the mean for the entire m. sartorius.

The sum of the forces produced by the stimulation of L 5 and L 6 is with a few exceptions slightly larger than the force produced by the stimulation of the

femoral nerve. This might be considered as an indication of bisegmental innervation of a small part of the muscle fibers. However, since the stimulation of the femoral nerve concluded the experiment, it seems more likely that this is due to fatigue of the muscle and deterioration in the state of the animal. It seems certain that bisegmental innervation if present at all is insignificant in the m. sartorius.

TABLE 2

Comparison of the forces (in grams) developed by the right and left sartorius and quadriceps muscles during stimulation of L 5 in normal rabbits

NO.	M. SARTORIUS			M. QUADRICEPS		
	Left	Right	Q.	Left	Right	Q.
17	64	34	+0.9	20	16	+0.3
18	7	22	−2.1	—	11	—
19	83	67	+0.2	43	48	−0.1
20	21	30	−0.4	13	15	−0.2
21	50	31	+0.6	48	25	+0.9
22	63	35	+0.8	58	26	+1.2
23	130	124	0.0	136	143	−0.1
24	13	11	+0.2	8	5	+0.6
25	7	14	−1.0	10	16	−0.6
26	35	57	−0.6	32	21	+0.5
27	94	81	+0.2	142	55	+1.6
28	58	75	−0.3	19	36	−0.9
29	55	18	+2.1	8	10	−0.3
30	70	20	+2.5	18	8	+1.3
31	22	25	−0.1	11	7	+0.6
32	40	21	+0.9	5	8	−0.6
33	36	32	+0.1	—	6	—
34	52	63	−0.2	73	97	−0.3
35	42	63	−0.5	92	45	+1.0
36	108	103	0.0	75	154	−1.1
37	30	72	−1.4	42	80	−0.9
38	8	10	−0.3	—	—	—
39	131	141	−0.1	33	73	−1.2
40	25	19	+0.3	13	—	—
41	14	36	−1.6	—	9	—
42	41	20	+1.1	—	—	—
43	44	50	−0.1	—	16	—
Means	51	47	+0.05	43	39	+0.09
Standard errors	7	7	0.19	9	9	0.19

Since in the following pages the forces developed in the right and left m. sartorius during stimulation of the connection between L 5 and L 6 will be compared under experimental conditions, it is necessary to be informed about the normal variations between the right and left side. This comparison has been carried out in a group of 27 normal rabbits; the results are collected in table 2.

Though the forces developed in the individual experiments vary greatly due to the segmental variation, the means of the left and right side do not differ significantly from each other or from this value found in the group of rabbits of table 1.

In order to exclude the influence of the great segmental variations, a way was sought to compare the two sides, which is independent of the absolute forces developed. For this purpose has been used the quotient (Q) of the difference between the forces developed in the left and right sartorius on stimulation of L 5 and the force of either the right or left sartorius, whichever is the smallest, thus

$$\frac{F_{(\text{left})} - F_{(\text{right})}}{F_{(\text{the smaller of left or right})}}$$

This quotient is positive when the force on the left side is the greater, negative when the force on that side is the smaller of the two. The mean of the Q's for the sartorius muscles reported in table 2 is + 0.05, its standard error is 0.19.

The m. quadriceps is a large and complicated muscle, like the m. sartorius, innervated by fibers from the 5th and 6th lumbar segment which reach the muscle through the femoral nerve. In table 2 the results are collected of a number of experiments in which the force developed in the muscle was recorded during the stimulation of the connection between L 5 and L 6. These forces vary greatly in the individual experiments; in several instances L 5 did not seem to participate in the innervation of the m. quadriceps even if in these animals the stimulation of L 5 caused a contraction in the m. sartorius. The mean force was about 40 grams, and it can be concluded that the 5th lumbar segment innervates only a small fraction of the large and powerful quadriceps muscle. The means of the force developed on the right and left side during the stimulation of the fibers from L 5 are not significantly different, the mean of the Q's is +0.09 with a standard error of 0.19.

It thus can be concluded that there is neither in the m. sartorius nor in the m. quadriceps a systematic difference between the right and left side, in the force developed by the stimulation of the motor fibers from L 5.

The force of contraction in the m. sartorius and m. quadriceps two months after partial denervation. As was shown the 6th lumbar spinal nerve supplies the majority of the motor fibers for the m. quadriceps and in most animals also for the m. sartorius. The removal of L 6 thus will produce a severe paresis in these muscles. In order to prevent regeneration, the 6th lumbar nerve was pulled out of the spinal cord, which can be done by a ventral approach as follows. After opening the abdomen the femoral nerve is exposed and traced cranially till the place where it is formed by the bundles from L 5 and L 6. The contribution from L 6 is clamped in a fine hemostat and the nerve is slowly and carefully pulled out to avoid damage to the connection between L 5 and L 6. In the nervous structure thus obtained the anterior and posterior root as well as the spinal ganglion can be distinguished. That the roots are removed completely is shown when the spinal canal is opened.

TABLE 3

Comparison of the forces (in grams) developed in the right and left sartorius and quadriceps muscles during stimulation of L 5 two months after the removal of L 6 on one side

In some of the animals the surface of the fiber cross section (in 1/1000 mm.²) of the left and right sartorius muscle has been compared

NO.	OPERATED SIDE	M. SARTORIUS						M. QUADRICEPS		
		Force			Fiber surface			Force		
		Operated	Control	Q.	Operated	Control	Q.	Operated	Control	Q.
44	R	98	23	+3.3				—	15	—
45	R	245	48	+4.1				318	41	+6.8
46	L	115	17	+5.8	1.26	1.19	+0.1	—	—	—
47	L	48	17	+1.8				8	8	0.0
48	L	180	61	+2.0				—	34	—
49	L	277	116	+1.4	0.87	0.55	+0.6	198	96	+1.1
50	L	130	67	+0.9	1.81	1.39	+0.3	—	—	—
51	R	16	23	−0.4				14	42	−2.0
52	R	166	77	+1.2	2.42	1.34	+0.8	39	158	−3.1
53	L	125	24	+4.2				—	—	—
54	L	176	54	+2.3	1.62	1.10	+0.5	—	—	—
55	R	217	38	+4.7	1.63	1.44	+0.1	116	35	+2.3
56	L	145	43	+2.4				107	17	+5.3
57	L	160	43	+2.7	1.79	1.39	+0.3	38	16	+1.4
58	R	207	67	+2.1	1.16	0.74	+0.6	330	204	+0.6
59	L	106	54	+1.0				39	17	+1.3
60	R	82	19	+3.3				20	7	+1.9
61	R	157	24	+5.5				52	6	+7.7
62	L	124	43	+1.9	1.16	1.05	+0.1	132	10	+12.2
63	L	233	68	+2.4				69	28	+1.5
64	L	91	30	+2.0	1.31	0.72	+0.8	9	36	−3.0
65	R	208	18	+10.6	1.64	0.96	+0.7	53	21	+1.5
66	R	60	33	+0.8	3.06	1.27	+1.4	—	12	—
67	L	50	13	+2.8	1.89	0.80	+1.4	8	9	−0.1
68	R	98	20	+3.9	0.80	1.32	−0.6	14	29	−1.1
69	R	131	33	+3.0				41	10	+3.1
70	L	196	21	+8.3				21	8	+1.6
71	L	142	24	+4.9	1.40	0.65	+1.1	—	—	—
72	R	233	52	+3.5				256	50	+4.1
73	L	96	9	+10.7				—	—	—
74	L	217	102	+1.1	1.07	0.99	+0.1	123	39	+2.2
75	R	153	16	+8.6	2.43	0.99	+1.5	—	5	—
76	R	70	28	+1.5				137	102	+0.3
77	R	95	46	+1.1	1.77	0.70	+1.5	133	54	+1.5
Means..........		143	40	+3.4	1.62	1.03	+0.6	95	40	+2.0
Standard errors.		11	4	0.5	0.14	0.07	0.1	20	9	0.5

In a number of animals the 6th and usually also the 7th spinal nerve was removed in this way on either the right or left side, leaving only L 5 intact as the sole motor innervation of sartorius and quadriceps muscles. Two months

later the tendons of these muscles were exposed and prepared for recording. The connection between L 5 and L 6 was exposed on both sides and stimulated. The forces thus recorded in 34 experiments are collected in table 3. At the time of the final experiment the age and weight of the animals were comparable with those of the control rabbits of tables 1 and 2. In all but one experiment the force developed by the m. sartorius on the operated side was larger than that on the normal control side. The mean of the forces developed in the m. sartorius on the non-operated side was 40 grams, whereas on the operated side the much larger mean force of 143 grams was found.

In order to express the relation between the forces of the mm. sartorius on both sides independently of the segmental variation, the quotient (Q) was computed of the difference in force of the operated and control side and the smaller of the two, which in all but one of these experiments was the force on the normal side, thus

$$\frac{F_{(\text{operated})} - F_{(\text{control})}}{F_{(\text{smaller of the two})}}$$

A positive Q thus signifies a gain in force on the operated side. The mean of these Q's is + 3.4, with a standard error of 0.5, a statistically highly significant increase. The forces produced on the operated side thus were on the average 4.4 times as large as those produced by the stimulation of the same nerve on the normal side. This large increase is the more remarkable since the experimental errors would act in the opposite direction. Damage to the connection between L 5 and L 6 either during the initial operation or during the dissection in the final experiment, which is not always easy because of the development of scar tissue, tends to decrease the muscle force developed on the operated side. Also the use of stimuli of too great intensity can increase the force developed on the normal side by the stimulation of elements of L 6, which have long degenerated on the operated side.

A similar though smaller increase of the force developed on the operated side was found in the m. quadriceps. The mean of the force developed on the control side was 40 grams, which is not different from the value found in the control series. On the operated side the mean force was 95 grams with the large standard error of 20 grams. Though in most experiments the force developed in the m. quadriceps on the operated side was the largest, in 5 out of 24 experiments the force on the control side was greater. The mean of the Q's was 2.0 with a standard error of 0.5. Though this increase is smaller than that found for the m. sartorius, it still is highly significant.

The course of the increase in force of paretic muscles. It was found that 2 months after the removal of L 6 the force developed in the m. sartorius by stimulation of the fibers from L 5 was 4.4 times as large as on the control side. The question can be asked, at what time does this increase start and does it still continue after the first two months. To investigate this a number of animals were prepared as described before and the forces developed in the sartorius and quadri-

ceps muscles by stimulation of the fibers from L 5 were recorded after 2 weeks, 1 month and 6 months.

After two weeks (table 4) the mean of the forces of the mm. sartorius and quadriceps on the operated side were slightly greater than on the control side. Also the mean of the Q's showed a slight gain on the operated side. However, these increases are statistically not significant.

After one month (table 5) the mean force developed in the m. sartorius on the operated side is 109 grams, on the control side 45 grams. Q is + 2.6 with a standard error of 0.7 and thus at that time there exists a highly significant increase in force of this muscle on the operated side. In this series the m. quadriceps on the operated side had hardly increased at that time.

TABLE 4

Comparison of the forces (in grams) developed in the right and left sartorius and quadriceps muscles during stimulation of L 5 two weeks after the removal of L 6 on one side

NO.	OPERATED SIDE	M. SARTORIUS			M. QUADRICEPS		
		Operated	Control	Q.	Operated	Control	Q.
78	R	119	65	+0.8	125	48	+1.6
79	L	24	59	−1.5	33	29	+0.1
80	L	31	22	+0.4	5	7	−0.4
81	R	55	16	+2.4	41	9	+3.6
82	L	91	28	+2.3	85	21	+3.0
83	R	73	21	+2.5	66	12	+4.5
84	L	143	170	−0.2	250	113	+1.2
85	R	8	15	−0.9	23	46	−1.0
86	L	58	74	−0.3	48	126	−1.6
87	L	7	7	0.0	—	—	—
88	L	103	83	+0.2	33	47	−0.4
89	L	6	17	−1.8	5	18	−2.6
90	R	95	43	+1.2	74	19	+2.9
91	R	76	57	+0.3	85	62	+0.4
Means		64	48	+0.4	67	43	+0.9
Standard errors		12	11	0.4	18	11	0.6

After two months, as has been shown (table 3), the increase in the m. sartorius is considerably larger than after one month, the Q being + 3.4. Also at this time a significant increase in the force of the m. quadriceps on the operated side was found (Q was +2.0).

After 6 months (table 6) a further increase in force was found. The mean of the force developed by the sartorius on the operated side was 192 grams, on the control side this was 49 grams. Q was + 5.2 with a standard error of 1.2. Also the mean force developed in the m. quadriceps on the operated side (145 grams) as well as Q (+3.3) were larger than after two months.

The mean of the forces developed in the control muscles of the various groups

Comparison of the forces (in grams) developed in the right and left sartorius and quadriceps muscles during stimulation of L 5 one month after the removal of L 6 on one side

In some of the animals the surface of the fiber cross section (in 1/1000 mm.²) of the left and right sartorius muscle has been compared

NO.	OPERATED SIDE	M. SARTORIUS						M. QUADRICEPS		
		Force			Fiber surface			Force		
		Operated	Control	Q.	Operated	Control	Q.	Operated	Control	Q.
92	R	178	28	+5.4	0.92	0.88	+0.1	35	—	—
93	L	186	155	+0.2				120	64	+0.9
94	L	55	39	+0.4				9	27	−2.0
95	L	105	48	+1.2				62	39	+0.6
96	L	183	108	+0.7	1.38	1.35	0.0	19	88	−3.6
97	L	133	12	+10.1	1.01	0.79	+0.3	7	7	0.0
98	R	143	29	+4.0	1.23	0.51	+1.4	20	44	−1.2
99	R	50	7	+6.1				35	19	+0.8
100	L	39	45	−0.2				8	37	−3.6
101	L	133	55	+1.4	1.66	1.28	+0.3	81	20	+3.1
102	R	77	17	+3.5	1.26	0.87	+0.4	15	8	+0.9
103	L	43	17	+1.5				7	11	−0.6
104	R	25	7	+2.6				7	5	+0.4
105	R	133	44	+2.0	0.85	0.50	+0.7	111	29	+2.8
106	R	155	80	+0.9	1.07	0.50	+1.2	138	41	+2.4
107	L	103	35	+1.9	1.54	1.09	+0.4	13	17	−0.3
Means.........		109	45	+2.6	1.21	0.86	+0.5	43	30	+0.04
Standard errors.		14	10	0.7	0.09	0.11	0.2	11	6	0.5

TABLE 6

Comparison of the forces (in grams) developed in the right and left sartorius and quadriceps muscles during stimulation of L 5 six months after the removal of L 6 on one side

In some of the animals the surface of the fiber cross section (in 1/1000 mm.²) of the left and right sartorius muscle has been compared

NO.	OPERATED SIDE	M. SARTORIUS						M. QUADRICEPS		
		Force			Fiber surface			Force		
		Operated	Control	Q.	Operated	Control	Q.	Operated	Control	Q.
108	R	86	22	+2.9				14	7	+1.0
109	L	113	55	+1.1				21	61	−1.9
110	L	213	37	+4.8	2.87	0.79	+2.6	311	20	+14.1
111	L	352	136	+1.6				216	67	+2.2
112	R	123	47	+1.6	2.22	0.67	+2.3	—	11	—
113	L	235	23	+9.2	1.30	1.23	+0.1	193	66	+1.9
114	L	240	41	+4.9				95	14	+5.8
115	R	227	24	+8.5	3.48	1.11	+2.2	109	35	+2.1
116	R	203	18	+10.3				125	—	—
117	L	68	15	+3.5				—	11	—
118	R	213	14	+14.2	3.23	1.26	+1.6	—	7	—
119	L	212	167	+0.3				218	115	+0.9
120	R	214	37	+4.8	2.32	2.00	+0.2	—	32	—
Means..........		192	49	+5.2	2.57	1.18	+1.5	145	37	+3.3
Standard errors.		21	13	1.2	0.32	0.19	0.5	33	10	1.7

remains approximately the same, but as time proceeds, the force of the muscles on the operated side increases very considerably. The force developed in the m. sartorius after 6 months is even larger than developed in the control sartorius muscle by stimulation of the n. femoralis (table 1). Figure 1, in which the changes in force in the m. sartorius are plotted against time, shows that the increase occurs most rapidly during the first months and that after 6 months no rapid changes are to be expected.

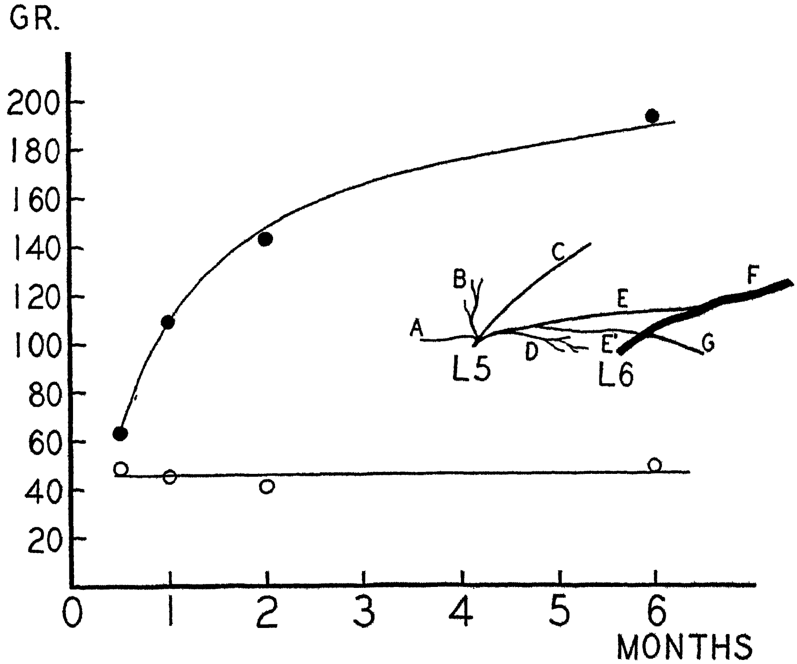

Fig. 1. The mean force developed in the sartorius muscles on the operated (dots) and control (circles) side, 2 weeks, 1 month, 2 months and 6 months after unilateral partial denervation, has been plotted against time.

The insert shows the formation of the rabbit's femoral nerve from contributions of L5 and L6. *A*, connection between the 4th and 5th lumbar spinal nerve. *B*, muscle branch. *C*, n. cutaneous femoris lateralis. *D*, muscle branch. *E*, contribution for the femoral nerve from L5. *E'*, a second connection between L5 and L6, which joins *G*, the connection between L6 and L7. *F*, femoral nerve.

Action potentials of partially denervated muscles. In 16 animals in which the 6th and 7th spinal nerve had been removed 2 months before, the action potentials of the m. sartorius during stimulation of L 5 were recorded on the operated and on the control side. The amplification used was equal on both sides and invariably the action potentials on the operated side were found to be larger than those on the control side. In experiment 55 (table 3) for instance the sartorius muscle produced an action potential of 1.7 mV. on the control side and of 10.9 mV. on the operated side. In rabbit 58 these values were 5.0 mV. and 12.5 mV. respec-

tively, etc. The mean Q for this group of 16 experiments was +4.4 with a standard error of 1.0.

Morphological findings in the sartorius and quadriceps muscles. It was noticed that in animals, in which the 6th and 7th spinal nerve had been removed 6 months before, the m. sartorius on the operated side appeared considerably broader and thicker than on the control side. This was also true, though to a lesser extent, in many of the rabbits which were kept for only two months.

The histological investigation of the m. sartorius, partially denervated by the removal of the 6th spinal nerve 2 months before, revealed in the majority of the experiments remarkably little change, an observation reminiscent of Exner's experience with the m. cricothyroideus. The cross section looked perfectly normal on superficial examination in all cases in which the sartorius muscle on the operated side developed a force of more than about 100 grams, irrespective of the size of the original innervation of the muscle by L 5. For instance, figure 2 B shows a cross section of a sartorius muscle (expt. 65) which developed 2 months after the removal of L 6 a force of 208 grams, whereas on the control side the stimulation of the connection between L 5 and L 6 developed a force of only 18 grams, indicating that in this animal L 5 innervated originally only a small part of the sartorius muscle. Nevertheless the histological appearance of the muscle was quite similar to that of a normal muscle (fig. 2 A) and throughout the entire preparation there was no obvious evidence of degeneration. In many of these muscles even the close scrutiny of the preparation with high magnification fails to show more than an occasional degenerated muscle fiber. In some instances, however, one or more bundles of degenerated fibers can be found, as is shown in figure 2 D. In this experiment (75) the force on the operated side was 153 grams, on the normal side only 16 grams. All experiments in which the force developed on the control side was larger than 30 to 40 grams, indicating a larger original participation of L 5 in the innervation of the m. sartorius, yielded preparations in which degeneration was practically absent. A difference between the operated and the control side sometimes noted was a certain irregularity of the muscle pattern, caused especially by a greater variability in the size of the muscle fibers on the denervated side.

In the preparations of muscles on the operated side which developed forces of less than 100 grams, more extensive bundles of degenerated fibers were present. In the few experiments in which the forces developed on the operated side were 50 grams of less, whole areas of degenerated fibers were found with bundles of functional fibers in between.

Obvious degeneration was also lacking in the majority of the preparations of sartorius muscles from animals in which the 6th lumbar spinal nerve was removed 6 months before. However, examination with high magnification revealed in these preparations also the presence of occasional degenerated fibers. The variability of the fiber diameter is in many of these preparations more pronounced than in the 2 months group (fig. 2 C). Several of the preparations showed a definite increase of the connective tissue in the muscle. The average diameter of the muscle fibers was in most of these experiments considerably larger on the oper-

ated than on the control side, which explains the increase in width and thickness of the whole m. sartorius mentioned before (compare fig. 2 A and 2 C).

The experiments in which the 6th spinal lumbar nerve was removed 1 month before, yielded preparations in which again no obvious degeneration was noticeable when the force developed by the m. sartorius was relatively large, whereas in the preparations of the sartorius muscles which developed less than about 100 grams frank degeneration was present.

Denervation of a muscle for two weeks does not produce marked degeneration, the fibers at that time are somewhat thinner, and darker stained than the normal ones. In the sartorius muscles which were obtained two weeks after the removal

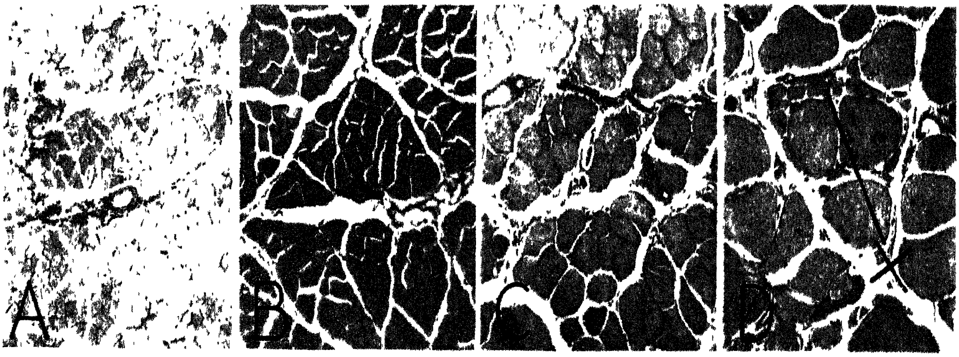

Fig. 2. *A*, cross section of normal sartorius muscle (Magn. 53 ×). *B*, cross section of sartorius muscle, partially denervated 2 months before (expt. 65). The force developed on the operated side on stimulation of L5 was 208 grams, on the control side 18 grams. There is no obvious degeneration (Magn. 53 ×). *C*, cross section of sartorius muscle partially denervated 6 months before (expt. 115). The force developed on stimulation of L5 was 227 grams on the operated, 24 grams on the control side. Note the large surface of the fiber cross section, as compared with that of the control muscle *A* (Magn. 53 ×). *D*, cross section of sartorius muscle partially denervated 2 months before (expt. 75). Stimulation of L5 developed a force of 153 grams on the operated, 16 grams on the control side. The picture shows one of the three bundles of degenerated fibers, *X*, found on the cross section (Magn. 131 ×).

of L 6 usually many small and darkly stained fibers were found, even in muscles which developed considerable force.

In suitable preparations the average surface of the fiber cross section was determined in the m. sartorius on the operated and on the control side. Though there are considerable individual differences, the mean of the fiber cross section surface on the normal side shows a slight increase when the one, two and six months experiments are compared. This is probably due to age differences of these groups of animals, since the removal of the 6th spinal lumbar nerve was always performed in rabbits of 1.5 to 2 kgm. weight.

With only one exception the fiber cross section surface on the operated side was larger than on the control side, in each of the one, two and six months experiments examined. In the same way as for the force developed, the Q of the fiber surface was computed. This value gives information on the relation of the surfaces on

the experimental and control sides, independent of the absolute values. A comparison of the mean Q of the one, two and six months experiments shows that this quotient increases as the interval between the removal of L 6 and the final experiment increases. In the one month group Q was 0.5 with a standard error of 0.2, in the two months group these figures were 0.6 and 0.1, and in the 6 months group 1.5 and 0.5. This indicates that though the fiber cross section surface increases on the control side with the interval between the removal of L 6 and the final experiment, the surface on the operated side increases considerably more.

The small forces developed in the quadriceps muscle 2 and 6 months after the removal of the 6th lumbar spinal nerve indicate that this large and strong muscle has not become completely re-innervated. Indeed in all preparations of that muscle large areas of degenerated fibers are present. The m. sartorius is a very suitable muscle for histological examination after denervation, since as was shown by Adrian (1925) for the m. tenuissimus and by Cooper (1929) for the m. sartorius, the motor unit is spread over the entire length of such a long and thin muscle, a cross section thus will always hit part of all motor units. This is not the case with the pennated m. quadriceps in which a cross section at an arbitrary level will reveal only part of the motor units. It is for this reason that in many of the preparations of the quadriceps which, as indicated by the effects of stimulation of the femoral nerve, contained active units, none was found on the cross section. In the preparations which showed functional muscle tissue the same variation in the size of the muscle fibers was noted as previously described in the m. sartorius.

Discussion. Two mechanisms can be considered to explain the increase in the force of the paretic m. sartorius; an increase in the diameter of the muscle fibers, and an increase in the size of the motor units by a more luxuriant terminal branching of the remaining motor nerve fibers and adoption of muscle fibers which originally were innervated by L 6. The a priori, very slight possibility that new motor nerve fibers would grow out from the spinal cord complementing the innervation of the m. sartorius, is excluded by the speed with which the re-innervation takes place, the process starting after two weeks and being well under way after one month.

The first mechanism cannot explain the sometimes very large increases observed. For instance in the 2 months experiments it was found that the mean Q for the surface of the fiber's cross section was 0.6 whereas the mean Q for the muscle force was 3.4. This means that whereas the mean fiber surface of the operated side was 1.6 times as large as on the normal side the force developed on the operated side was 4.4 times as great as on the normal side. Thus, even assuming that the force developed per fiber is directly proportional to the surface of its cross section[2] there is still $\frac{4.4}{1.6} = 2.7$ times not accounted for, and this increase must be caused by the second factor, the increase in size of the motor unit.

[2] The influence of the increase in fiber cross section surface is probably much smaller than here assumed, since experimental evidence has been collected which shows that the increase of the force in a muscle thus hypertrophied falls far short of its increase in fiber cross section surface.

The importance of the growth of the motor unit is strongly supported by the result of the histological investigation of the sartorius muscle.

It must be concluded that of the two changes which occur in the paretic muscle tending to repair its function, the major factor is growth in the size of the motor unit, with, as an additional factor, the hypertrophy of the individual muscle fibers.

The growth of the motor units occurs mainly during the first months after the partial denervation as is indicated by the usually practically complete re-innervation of the m. sartorius in the 2 months experiments. The increase in fiber thickness seems to continue much longer and may be mainly responsible for the increases in force after that time.

If by growth of the motor units the muscle is completely re-innervated and if on top of this, fiber hypertrophy occurs, the resulting muscle may become stronger than the entire control muscle. Indeed, in the 6 months series the mean force of the m. sartorius on the operated side during stimulation of the fibers from L 5 was larger than the mean force of this muscle in the control animals (table 1) during stimulation of the entire femoral nerve. It should be emphasized that in the other muscles of the leg, for instance in the m. quadriceps, these two factors are not able by far to make up for the loss in force incurred by the destruction of L 6.

We have seen that the participation of L 5 in the innervation of the sartorius is quite variable. In a sartorius which is almost fully innervated by L 5 the motor units will be able to grow only slightly after the removal of L 6, since in that case there are present only a small number of denervated muscle fibers, which can be adopted by the remaining motor units. On the other hand in the sartorius in which L 5 innervates only a small part of the muscle the opportunity for growth of the intact units will be large. It is thus to be expected that Q is greatly influenced by the size of the original contribution of the 5th lumbar segment. The theoretical relation between Q and the original contribution of L 5 is given in the line of figure 3 for a muscle developing 150 grams when fully contracting, assuming that it becomes completely re-innervated. In the same graph the Q's determined in the 2 and 6 months experiments have been plotted against the force developed during stimulation of L 5 on the control side. It is obvious that the largest increases are found in the animals in which L 5 originally innervated only a small part of the sartorius muscle. Though the variations are considerable, the Q's follow the general trend of the theoretical line in this figure computed for a sartorius muscle of average strength. The relation between the increase of force and the original innervation of the sartorius by L 5 is one of the strongest arguments for the importance of the increase in the size of the motor unit as a factor in the improvement of the paretic m. sartorius.

It is obvious from the relation between Q and the original innervation by L 5 just mentioned, that the mean Q is a poor indicator of the potentialities of the motor unit growth. In order to evaluate this potentiality we should consider only those experiments in which the original innervation of the m. sartorius by L 5 was small. From figure 3 it appears that, even allowing for the factor of muscle fiber hypertrophy, a tenfold increase in the size of the motor unit is prob-

ably not uncommon in the m. sartorius when the conditions for such an increase are favorable. In the m. quadriceps which never becomes completely innervated no significantly larger Q's were found. However, it is not certain that a tenfold increase is the limit of the potentiality of the motor unit growth, since it is possible that in the m. quadriceps the distribution of the connective tissue in the muscle prevents a further growth of the motor unit, whereas in the m. sartorius the smallness of the muscle is the limiting factor.

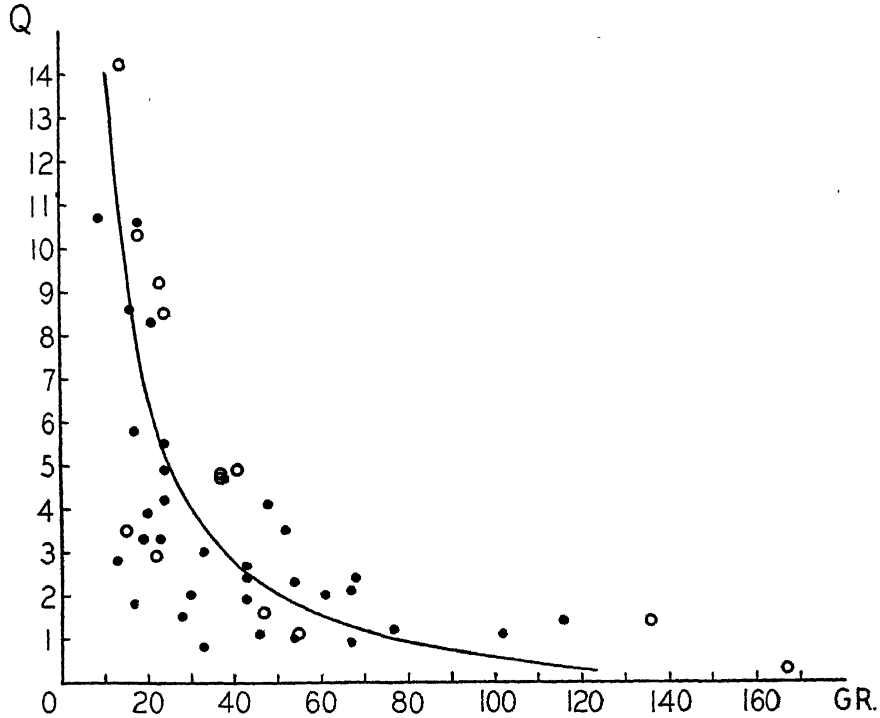

Fig. 3. The Q's computed from the forces developed in the operated and control sartorius muscles in the 2 (dots) and 6 (circles) months experiments, have been plotted against the forces developed in the control muscles on stimulation of L5. The line shows the theoretical relation between these two values for a muscle developing a force of 150 grams when fully contracting, assuming that the muscle becomes completely re-innervated.

The mechanism of the increase in force observed above in paretic muscles may well be comparable with that of the improvements seen in paretic muscle of long standing after forceful massage (Billig, van Harreveld and Wiersma). The latter have been explained on the basis of an injury to the finest intramuscular branches of the motor nerve fibers leading to increased ramification and muscle fiber adoption during the ensuing process of regeneration. It is not beyond conjecture that the paretic leg of the rabbit is subjected to mild injuries as the animal moves around, and that these would be sufficient to produce the damage of the intramuscular nerve fibers. If this is the basis of the reinnervation then a ready expla-

nation for the greater increases in the m. sartorius as compared to the m. quadriceps can be seen in the superficial location of the m. sartorius, since the remaining motor units of the m. quadriceps, hidden in the substance of the muscle, are less subject to these slight injuries.

A possibility which should not be overlooked is that the damage to the intramuscular ramification is caused, not by external injury, but by the contraction of the intact motor units, displacing their fibers with respect to the denervated ones. It has been found that this mechanism can cause extensive damage to the intramuscular ramification in the crustacean muscle (van Harreveld, 1939).

Another possibility is that the re-innervation of the m. sartorius is comparable with the spontaneous re-innervation of areas which have lost their sensory nerve supply, from the surrounding intact nerves. Speidel (1935) found this in tadpole tails, and Weddell, Guttmann and Gutmann (1941) found that the shrinking of an anesthetic area, even when no regeneration of the old sensory pathway occurs (Pollock, 1920), is due to an outgrowth of nerve fibers from the surrounding sensory nerves. The growth of the motor units observed in the m. sartorius thus might be the motor counterpart of Weddell, Guttmann and Gutmann's observation on the sensory re-innervation of the skin.

The growth of the motor units observed is of considerable practical interest, since it shows that paretic muscles can improve by other mechanisms than by the regeneration of the lost nervous structure. For instance, the paresis after the acute phase of poliomyelitis tends to improve very considerably, even to apparent normalcy, during the first ½ to 1 year after the attack. Since the nerve cells destroyed during the acute phase are not replaced, it is unlikely that this improvement is caused by nervous regeneration, and it is likely that the growth of the remaining motor units is mainly responsible for the improvements. This consideration leads to the expectation that muscles which have been paretic due to poliomyelitis and which have apparently returned to normalcy are in fact profoundly changed and consist of relatively few but large motor units.

I am greatly indebted to Mrs. J. Wiersma and Miss R. E. Estey for valuable assistance.

SUMMARY

1. A severe paresis is produced in the m. quadriceps and usually also in the m. sartorius of the rabbit by pulling the 6th lumbar spinal nerve out of the spinal cord. The small contingent of motor fibers from L 5 forms the sole remaining motor innervation of these muscles.

2. Recording the forces developed by the right and left mm. sartorius and quadriceps during the stimulation of the fibers from L 5, a few months after the removal of the 6th lumbar spinal nerve, it was found that the force produced on the operated side was considerably larger than on the control side.

3. This increase in muscle force starts about two weeks after the removal of L 6, it is considerable during the first few months and has probably not quite ceased after 6 months.

4. No serious or obvious degeneration was usually found in the m. sartorius two months after the removal of L 6, even in the instances in which this muscle was innervated originally only to a small extent by L 5 (as indicated by the small forces developed on the control side during stimulation of L 5).

5. The average surface of the muscle fiber cross section in the m. sartorius on the operated side was found to be larger than on the control side.

6. It was concluded that the increase in force observed a few months after the partial denervation of the sartorius and quadriceps muscles is due mainly to an adoption by the motor units belonging to the 5th lumbar segment, of muscle fibers innervated originally by L 6. This adoption would be made possible by an increase of the branching of the end ramification of the motor fibers of L 5. It is possible that the increase in thickness of the muscle fibers is an additional factor in the increase of muscle force of the partially denervated muscles.

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FACTORS CONCERNED WITH THE INDUCTION OF TOURNIQUET SHOCK

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Following severe hemorrhage in animals, a hyperglycemia occurs which differs from that produced by adrenalin in two respects; it is more marked and is not influenced by denervation of the carotid sinuses (1). During shock produced by the application of limb tourniquets, there is also a moderate elevation of blood sugar. As in the case of hyperglycemia following hemorrhage, it has been of interest to study the magnitude of the blood sugar rise in tourniquet shock and the effect on it of carotid sinus denervation. In the course of these studies other changes were also observed that might be interpreted, together with the hyperglycemia, as manifestations of general autonomic nervous system stimulation. The presentation that follows deals not only with the blood sugar changes in normal dogs and dogs with denervated carotid sinuses following the application or release of limb tourniquets, but also describes alterations observed on the effect of anesthesia, on cardiac rate, on hematocrit, on blood pressure, on splenic contraction, and on the electrocardiogram during the tourniquet procedure. Variations in the picture of the shock produced and local circulatory changes in the occluded limbs are correlated with variations in the length of time of tourniquet application. A few studies of electrolytes have been made where they seemed pertinent.

Mechanisms concerned in the hyperglycemia of tourniquet shock. A total of thirty-four dogs was used. Seventeen were normal controls; in the other seventeen bilateral carotid sinus denervation had been completed seven to fourteen days prior to the study of the influences of tourniquet application.

There was a rise of the blood sugar after release of the tourniquets in all but three of the normal animals. The increases ranged from 4 mgm. per cent to 83 mgm. per cent, with an average of 39 mgm. per cent. The highest values for seven of these dogs were terminal, from 60 to 160 minutes after release. In six others, the intermediate values between release and death were higher than the terminal, and only one determination after release was made for the fourteenth.

The three dogs with decreases showed 7, 10 and 35 mgm. per cent reduction respectively shortly before death, which occurred after 150 and 152 minutes for the first two, and 180 minutes for the third. The last animal was sacrificed because of poor condition.

Following release of the tourniquets, two of the sinus denervated dogs showed

[1] The work described in this paper was done with the aid of grants from the Commonwealth Fund and under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Yale University.

a slight and transient rise of 18 mgm. per cent and 19 mgm. per cent respectively. The other fifteen showed a fall varying between 4 mgm. per cent and 85 mgm. per cent. The lowest values were terminal with only two exceptions in which they were intermediate. The average survival time of these seventeen animals was 124 minutes.

After tourniquet release, there were no significant variations between the normal and the sinus denervated dogs, either in the steady decrease of blood pressure, venous oxygen, and carbon dioxide, or in the rise of the hematocrit and blood lactic acid. The first impression that the survival period was shortened by sinus denervation could not be sustained.

The fact that the blood sugar fell in sinus denervated dogs with release of the tourniquets, and in this way contrasted with controls whose blood sugar rose under the same circumstances, suggested that the adrenal-carotid sinus mechanism is concerned in the mild hyperglycemia of tourniquet shock. It will be recalled that this is not the case following hemorrhage.

In the course of the studies just described, the blood sugar usually had become elevated both in normal and sinus denervated animals before the tourniquets were released. Determinations, therefore, were made immediately before application of tourniquets and also two and one-half and five hours later.

The rise in blood sugar for the normal dog during the period of tourniquet application varied between 6 mgm. per cent and 97.6 mgm. per cent, and averaged 37 mgm. per cent for the seventeen animals of the group. Determinations also were made two and one-half hours after application and the values in eight animals were found to be even higher than after the five hour period.

In seventeen sinus denervated dogs the increase after five hours of ligation averaged 55 mgm. per cent and in nine animals of this group it was also at a higher level at the two and one-half hour period.

The absence of significant difference in the hyperglycemic reaction of normal and carotid sinus denervated animals during the period of tourniquet application, supports the conclusion that adrenalin is not causatively related to this rise in blood sugar and that the increase may be a result of sympathetic stimulation.

Stimulation of the autonomic nervous system by tourniquet application. Other evidences of sympathetic stimulation also were observed. They include influences of tourniquet application on anesthesia, cardiac rate, hematocrit, blood pressure and splenic contraction.

In the normal dog 25 mgm. of nembutal/kgm. of body weight injected intravenously has been found to result in satisfactory surgical anesthesia for one and one-half to two hours. This time was halved when tourniquets were applied and was further markedly reduced if animals with denervated sinuses were used. The slow respiratory rate after nembutal in the normal dog (8–12 a min.), was increased soon after tourniquet application to 30 a minute and still further in the sinus denervated animal. The same variations occurred in cardiac rate, the pulse rising from 120 per minute with the application of the tourniquet to 180–240 per minute. It only returned to the previous level after more than an hour for the normal dog. For the sinus denervated animal it rose to 300 or

more a minute, and also fell more gradually. Tourniquet application likewise increased the force of cardiac contraction observed by palpation in both groups of dogs.

Normal dogs showed only a very slight change in the hematocrit during the period of tourniquet application. The variation in eight animals ranged from an increase of 5 vol. per cent to a decrease of 3 vol. per cent with an average of +2 vol. per cent. In contrast, eight sinus denervated dogs all showed increases in hemoconcentration ranging from 9.5 vol. per cent to 19 vol. per cent and averaging 14.5 vol. per cent.

Blood lactic acid was determined before application of the tourniquets and again just prior to their release in nine normal and eighteen sinus denervated animals. Two of the animals of the first group showed a decrease of 2.3 and 5.2 mgm. per cent respectively; the other seven showed an increase varying from 1.9 to 23.8 mgm. per cent and averaging 11.1 mgm. per cent. Only one of the animals of the sinus denervated group showed no change. The other seventeen showed elevation of blood lactic acid varying from 8.5 to 46.3 mgm. per cent and averaging 28 mgm. per cent.

A rise of 20–30 mm. Hg in the mean systolic blood pressure promptly followed tourniquet application to one hind leg of a normal dog under nembutal anesthesia. A tourniquet applied to the second leg further elevated the pressure slightly. This elevated pressure persisted for one-half hour or more and then slowly returned to the pre-operative level. The rise in pressure coincided in time with the altered cardiac and respiratory rates and was as great as 50 mm. Hg in sinus denervated animals. In the sinus denervated dogs the rise in hematocrit was definite as mentioned above, while the controls showed neither significant rise nor fall. In both groups nembutal resulted in splenic dilatation. The stimulus in the normal dog was not adequate to counteract such effect, but with the sinus removed, the same stimulus resulted in splenic contraction and hemoconcentration. The corollary also held. With preliminary removal of the spleen in sinus denervated dogs, tourniquet application was not followed by significant hemoconcentration.

These findings during the period of tourniquet application involving blood sugar, cardiac contraction, pulse rate, blood pressure, hematocrit and splenic contraction pointed to sympathetic stimulation, as a result of pressure from the tourniquets. The fact that the signs all were more marked in sinus denervated animals was in accord with removal of the influences the sinus exerts on autonomic function (2).

Observations on the mechanism of sympathetic stimulation. Experiments were undertaken to determine whether pressure exerted by the tourniquets upon underlying nerves of the leg could be related to the sympathetic stimulation. Procaine infiltration of the skin and deep tissues of the leg in the area underlying the tourniquet were completed before its application and repeated at about twenty minute intervals. Local anesthesia of the limb thus induced eliminated all the general effects of the tourniquet, including those on respiration and pulse rate, blood pressure, blood sugar and hematocrit.

The same abolition of all tourniquet effects was obtained when the lumbosacral nerve roots had been cut before tourniquet application.

This evidence of the origin of the stimulation of the sympathetic system from pressure exerted by the tourniquet on underlying nerves, further suggested that the rise in blood pressure might be another expression of the same mechanism. The splenic contraction with resulting rise in the hematocrit, it was thought, might only be a part of the general contraction of the splanchnic vessels. Accordingly the following experiment was completed: After removal of the stomach, large and small intestine and spleen without change in blood pressure, the tourniquets were applied to the legs as usual. No rise in blood pressure resulted. The responsiveness of the animal at this stage was demonstrated by eliciting the normal reaction to intravenously administered 0.2 mgm. of adrenalin hydrochloride.

As has been noted, the signs of sympathetic stimulation after tourniquet application did not persist. To determine whether this failure of response resulted from injury to the nerve by direct pressure of the tourniquet or whether it was an expression of exhaustion of the mechanism, a tourniquet was applied to the second leg when the blood pressure, pulse and respiration had returned to normal. The interval between application of the first and second tourniquets was varied. It was found that the response was lacking, or insignificant when the elapsed time did not exceed twenty four hours. Beyond this, the application of a tourniquet to the second leg was followed by a rise in pressure. Subsidence of the stimulation was interpreted, therefore, as a manifestation of sympathetic fatigue, following excessive stimulation.

Local loss of fluid after tourniquet release in normal and sinus denervated dogs. There was a marked discrepancy between control animals and the sinus denervated group in the swelling of the legs following release of the tourniquets. After a five hour application, the hind legs of the normal dog promptly began to swell and within a short time this became very marked. The damage to the muscles readily became apparent when a severely shocked animal occasionally survived for sufficient time to allow necrosis to become manifest. Histological examination of the tissues of the leg, removed even as early as an hour after tourniquet release, showed an accumulation of red blood cell and fibrin-containing fluid in the interstitial tissues and also extensive swelling and some necrosis of the muscle fibers.

All of these phenomena were much less pronounced in similarly treated sinus denervated dogs. The swelling of the legs with the release of the tourniquets either was definitely decreased or absent. Histologically, exudation was scant with red blood cell accumulations rare and muscle degeneration far less apparent, only appearing as frank necrosis in occasional fibers.

The possibility that the extent of the muscle change might be expressed in increased plasma phosphate led to its determination in a series of normal and sinus denervated dogs before application of the tourniquets, after their release and just before death. No change in plasma phosphate occurred during the five hour period of tourniquet application. The rise in plasma phosphate after

release is illustrated in table 1. The results were inconclusive. This might have been anticipated as there are other important factors associated with shock, especially the decreased circulating blood volume, that elevate serum phosphate. The slightly smaller increase in plasma phosphate in the sinus denervated dogs is consistent with the much less extensive histological evidence of change in the muscle fibers of the extremities.

The effect of varying the time of tourniquet application on the local loss of fluid and the development of symptoms of shock. In the course of the foregoing studies on physiological changes associated with tourniquet shock, it was repeatedly observed that variation in the duration of tourniquet application led, after release, to very different results locally in the occluded limbs and also to differences in the time of appearance and severity of the ensuing symptoms of shock. Consequently, systematic study was undertaken of these differences after tourniquet application periods varying from sixty-five hours to two and one-half hours.

TABLE 1

Plasma phosphate

	NORMAL		SINUS DENERVATED	
	Before application of tourniquets	After release, terminal	Before application of tourniquets	After release, terminal
	mgm. %	*mgm. %*	*mgm. %*	*mgm. %*
1	6.5	22.6	6.8	24.0
2	5.2	26.6	4.5	12.5
3	4.6	33.4	6.1	17.4
4	3.75	20.4	4.7	24.3
5	3.5	19.3	4.5	17.0
6			3.9	17.0
Total..........	23.5	122.3	30.5	112.2
Average... ...	4.7	24.5	5.1	18.7
Phosphate increase: 19.8 mgm. %			Phosphate increase: 13.6 mgm. %	

1. *Local and systemic changes following release of tourniquets applied sixty-five or forty-eight hours.* Observations were made in sixteen animals with either one or two hind limbs occluded. During the application of the tourniquets, moderate swelling occurred. After release there was no increased swelling, but gangrene developed. Just prior to release the femoral arteries and veins of the sixty-five hour dogs were found to be occluded by thrombi. In the forty-eight hour group only the femoral veins were so occluded.

In both groups of animals, after release, the shock picture did not develop. The experiment was terminated forty-eight hours after the tourniquets were removed because of the condition of the legs.

2. *Local and systemic changes following release of tourniquets applied twenty-four hours.* Seven animals were studied, with one or both hind limbs occluded.

There was, as with the sixty-five and forty-eight hour dogs, moderate swelling of the legs during the period the tourniquets were in position and the legs became markedly swollen after release. No thrombi were found in any of the femoral arteries but in two instances the femoral veins were occluded. Perfusion of Tyrode's solution through some legs (femoral artery-femoral vein, if vessels were free) at a given pressure resulted in great swelling of the limbs and practically no return of the perfusate. Histologically, sections of the perfused limbs revealed enormous quantities of interstitial fluid separating the individual muscle bundles. Capillary thrombi were not present and the patency of the smallest vessels was further attested by the presence in them of India ink, if this had been added to the perfusate.

As in the two preceding groups of dogs, the twenty-four hour animals did not develop shock after release of the tourniquets.

3. *Local and systemic changes following release of tourniquets applied seventeen or eighteen hours.* Seven dogs were used with one or both hind limbs occluded. On release, there was great swelling of the limb immediately, and in limbs perfused with Tyrode's there was marked swelling and reduction in the return of perfusate. No thrombi in major vessels or capillaries were present. Histologically the edema of the soft tissues was marked.

The symptoms and signs of shock were studied in three of the seven animals in this group. Shock did not develop immediately and was never severe until just before death, which occurred between twenty and thirty hours after release of the tourniquets.

4. *Local and systemic changes following release of tourniquets applied nine hours.* Four dogs were used, with one hind limb occluded. Upon release of the tourniquets there was swelling of the limb. No observations on the presence of thrombi or the return of perfusate were made in this group.

In contrast to the seventeen or eighteen hour animals, marked signs of shock developed promptly in the nine hour dogs and three of four of these animals died within ten hours of release of the tourniquet.

5. *Local and systemic changes following release of tourniquets applied five hours.* Several hundred dogs were studied during these and previous investigations. The majority of these animals had both hind legs occluded. As already stated, upon release of the tourniquets there was immediate, marked swelling of the legs with interstitial edema of the soft tissues and some necrosis of muscle. Studies on plasma phosphate on six of these animals have been reported above. In limbs perfused with Tyrode's the volume of perfusate recovered was reduced, and the legs became definitely swollen.

Following release of the tourniquets applied five hours previously normal dogs like those in the nine hour group, promptly developed symptoms of shock (3).

6. *Local and systemic changes following release of tourniquets applied two and one-half hours.* Six dogs were studied with one or both hind legs occluded. After tourniquet release in this group there was no swelling of the legs and no interference with the return of perfused Tyrode's solution.

In contrast to the five hour dogs, after release of limb tourniquets in position two and one-half hours, shock did not develop, and the animals had completely recovered in a few hours.

The observations recorded for the above groups of animals warrant brief discussion. If the application of the tourniquet is maintained long enough, thrombotic occlusion of the major vessels of the limb occurs; thereafter, upon release, no appreciable local fluid loss can take place because of the blocked circulation; hence there is no further swelling of the limbs. Conversely, there can be only minor return to the systemic circulation of any product that may have accumulated in the limb tissues during occlusion. This fact may be correlated with the non-appearance of the shock syndrome after the longer periods of tourniquet application in which thrombi have been found in the femoral artery and/or veins.

Thrombi are usually not present in the major vessels or capillaries of the extremities after twenty-four hour and seventeen–eighteen hour tourniquet application. Consequently a blocked circulation cannot be responsible for the delayed appearance of the symptoms of shock and their mildness—definite observations in these groups of animals after tourniquet release. Local fluid loss (swelling of the legs) is very marked in these dogs, involving greatly increased capillary permeability. In the nine hour and five hour groups local fluid loss and local circulatory changes do not appear so promptly and are not quite as extensive. These dogs develop severe symptoms of shock promptly after tourniquet release. It is questionable whether the differences in speed of appearance and extent of local fluid loss and circulatory changes are great enough to explain the marked discrepancy in symptomatology following the release of tourniquets in position five and seventeen hours.

The production of shock without accompanying local fluid loss. From the above discussion it is clear that it would be of interest to attempt to dissociate the factors of local fluid loss and local circulatory changes in the production of shock from other factors as yet unknown. This problem was approached in the following way. From the studies described in the foregoing section, it was known that release of tourniquets after two and one-half hour application was not accompanied by local loss of fluid. Furthermore, studies on tourniquet shock, with only a very few exceptions, have been carried out at room temperature. The possibility of accelerating chemical change in the muscle without fluid loss during a short period of tourniquet application by warming the extremities, suggested itself. This idea was supported by the well-known fact that the severity of tourniquet shock is decreased when the extremities are refrigerated and also by isolated reports on the influence of heat (4, 5).

Accordingly after tourniquet application the legs of the test animals were immersed in water at 47°C. for two hours. The tourniquets were then released within ten minutes after removal from the bath. Twenty experiments of this variety have been completed with similar results in all. Shock developed within one-half to one hour after release and ended fatally within two to four hours. It should be emphasized that this picture of severe shock developed *without*

swelling of the legs during the period of survival in thirty seven of the forty legs. This was corroborated in many instances by histological examination. In the three exceptions, swelling was due to hemorrhage. Further, in a few instances, the legs were perfused with saline following the release of the tourniquets after two hour application at 47°C. No escape of the perfusate into the tissues was observed.

The short period involved in the individual experiments made it unlikely that bacterial toxins were concerned in the induction of shock (6). To secure direct information, animals pretreated with gas bacillus antitoxin and with both antitoxin and penicillin were studied. The results are presented in table 2 and together with bacteriological studies of blood and tissues made at the death of the animals, exclude infection as a cause of shock in this particular investigation.

Determinations of serum electrolytes. In view of the absence of specific information concerning the chemical nature of the toxic factor arising in the ligated legs and also of the known increase of serum electrolytes, potassium levels (7, 8) were determined in four experiments of this series. Samples of serum were secured, just prior to tourniquet release, forty minutes later when the blood pressure was approximately at shock level, and then at intervals of half hour until fatal termination. There was a rise in the potassium values from the pre-release level of 5 m. eq. to 8.5 $\pm$ 0.5 m. eq. when shock was evident and further increase to 9–9–12 and 12 m. eq. just before death.

Serum phosphates also were determined in these four animals as well as in many others. The values for this electrolyte likewise not only were increased but were tripled in the serum prior to death. Serum potassium and phosphate in such excess must be considered in the precipitation and the fatal outcome of shock. One measure of their toxicity is available through alteration in the electrocardiogram. Increase in the T wave, associated with depression of the ST interval and heart block, together with disappearance of the P wave, are expressions of toxic effects of potassium on the heart (9). Latent tetany, accompanying increase in serum phosphates leads to a marked change in cardiac action, expressed by the K value—a quotient of the length of the electric ventricular systole divided by the square root of the rate (10). Electrocardiographic studies were, therefore, made on three of the four above animals in which serum potassium and phosphate were determined. The report follows.[2]

Dog 1. The hind limbs were ligated for two hours and ten minutes in water bath at 47°C. As compared with the control tracing (ECG I), the significant changes are slight depression of the S-T segments in leads 2 and 3, and moderate increase in the size of T; the latter change was progressive only in one of the series. Auricular activity remained normal throughout, as did intraventricular conduction. The duration of ventricular systole, expressed in terms of "K", was 0.359 in the control ECG, and 0.329 in the tracing obtained just before death.

Dog 2. Hind limbs ligated for two hours and ten minutes in water bath at 47°C. As compared with the control tracing (ECG I), no definite changes in the level of S-T or in the

[2] The electrocardiograms were made and interpreted by Dr. Arthur J. Geiger of the Department of Internal Medicine. This assistance is gratefully acknowleged.

amplitude of T occurred in any of the subsequent ECGs, and auricular activity and normal QRS conduction persisted to the end. The duration of ventricular systole, expressed in

TABLE 2

Bacteriological studies on dogs with limbs ligated for two hours at 47°C.

DOG NO.	WT.	ANTITOXIN	PENICILLIN	REMARKS
	kgm.			
1	10	*P.D. 10,000 U. *B. Welchii* P.D. 10,000 U. *V. Septique* i.v. 2½ hrs. before tourniquet release ⊥.		No significant swelling or edema of legs on gross examination at autopsy. Survival time—4 hrs. 30 min.
2	9.2	†Lilly 20,000 U. Gas gangrene antitoxin i.v. 3½ hrs. before tourniquet release ⊥.		No edema of legs on dissection at autopsy. Survival time—2 hrs. 30 min.
3	9.8	†Lilly 20,000 U. Gas gangrene antitoxin i.m. 18 hrs. before tourniquet release. Repeated 2¾ hrs. before tourniquet release ⊥.		Musculature of legs dry. Survival time—2 hrs., 20 min.
4	9.6	†Lilly 20,000 U. Gas gangrene antitoxin. 6,000 U. tetanus (Lederle) i.m. 18 hrs. before tourniquet release ⊥.		Both thighs dry. Survival time—3 hrs. 40 min.
5	10.8	*P.D. 10,000 U. *Perfringens* P.D. 10,000 U. *V. Septique* P.D. 1,500 U. *Oedematiens* i.m. 24 hrs. before tourniquet release. Repeated 16 hrs. and 4 hrs. before tourniquet release ⊥.	1,300 U./kgm 4 hrs. before tourniquet release. Repeated 2 hrs. and ½ hr. before tourniquet release ⊥.	No aerobic or anaerobic growth from blood 30 min. after release or at death. None from muscle biopsy at death. Survival time 3 hrs. 30 min.
6	9.1	*P.D. 10,000 U. *Perfringens* P.D. 10,000 U. *V. Septique* P.D. 1,500 U. *Oedematiens* i.m. 20 hrs. before tourniquet release. Repeated 4½ hrs. before tourniquet release ⊥.	1,550 U./kgm 2½ hrs. before tourniquet release. Repeated ½ hr. before release ⊥.	No aerobic or anaerobic growth from blood and none from muscle biopsy. Dog's legs dry. Survival time 40 min.

* Parke Davis.

† Lilly: Antitoxin for dogs 1 to 4 received from manufacturer 3 months before use. Antitoxin for dogs 5 and 6 received from manufacturer 3 days before use.

terms of "K", was 0.353 in the control ECG and 0.384 in the tracing obtained just before death.

Dog 3. Hind limbs ligated for two hours and ten minutes and in water bath at 47°C. As compared with the control tracing (ECG I), the significant changes are slight depression of the S-T levels and moderate increase in the amplitude of T in leads 2 and 3. These changes are not progressive between ECG II and III during which interval of forty-five minutes the dog progressed into profound shock. In ECG IV, taken when the dog was in an agonal state and had ceased respiring, there is periodic auricular standstill, with occasional sinus discharges as well as nodal and ventricular escaped beats. The duration of ventricular systole, expressed in terms of "K" was 0.288 in the control ECG and 0.290 in ECG III.

COMMENT. The duration of ventricular systole, corrected for heart rate, became slightly shorter in the course of one experiment, slightly longer in the second and was virtually unchanged in the third; the relatively slight differences and inconsistency of trend argue against their being of significance.

The relatively slight S-T and T changes seen in the tracings following release of the tourniquets are consistent with early potassium effects (6–8 m. eq.). The periodic suppression of auricular activity in ECG IV, taken during the agonal moments of Dog 3, are consistent with potassium effects seen with serum levels of about 8 m. eq. The characteristic effects of late potassium poisoning, which include complete auricular standstill and intraventricular block, were not obtained during the survival period of these animals, and death occurred with respiratory arrest *before* cardiac arrest. The evidence indicates that death in these dogs was not due to potassium poisoning.

SUMMARY AND CONCLUSIONS

Hyperglycemia develops during the five hour period of tourniquet application to the legs of dogs and continues to be augmented after release in the animal with intact carotid sinuses.

When the carotid sinuses are denervated, hyperglycemia develops during the period of tourniquet application but is not increased after release.

It is concluded that the hyperglycemia after release of tourniquets, as distinct from the hyperglycemia developing during tourniquet application, is dependent upon increased adrenalin discharge.

The hyperglycemia that develops during the period of tourniquet application is accompanied by increased rate and strength of cardiac action and by elevated blood pressure. These signs are all more marked when the carotid sinuses are denervated preliminarily and they are interpreted as expressions of sympathetic stimulation.

The stimulation of the sympathetic system is a result of pressure exerted by the tourniquets upon underlying nerves, as can be demonstrated by their block with local anesthetics or by their section.

The stimulation of the sympathetic system is limited in time and is followed by a longer period of diminished reactivity.

Preliminary carotid sinus denervation does not change the outcome or the survival period after release of tourniquets applied to the legs for five hours. A noteworthy observation under these circumstances is the markedly diminished swelling of the legs of dogs with denervated sinuses.

When tourniquet application is sufficiently protracted, thrombi develop in the large vessels of the extremities and release is not associated with signs of shock.

The length of the period of tourniquet application, even when this is shortened to avoid thrombus formation, definitely influences the symptomatology that follows release. Symptoms of shock most marked with five hour application grow less not only when the period is shorter but also when it is longer.

No explanation is available for the lack of symptomatology with longer tourniquet application, when thrombi are absent.

Its absence with shorter periods of tourniquet application is more readily understood. No swelling of the legs occurs on release after two hours of ligation. Even when the extremities are heated in a bath at 47°C. during the two hour period of application, no swelling of the legs follows release. However, shock develops promptly, ends fatally in two to four hours, and is indistinguishable from the syndrome that follows release after five hour ligation at room temperature.

The possible rôle of infection in these two hour experiments at 47°C. has been excluded. The known increases in serum potassium and phosphate could not be shown to have major toxic action as determined by the electrocardiogram.

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ALKALINIZING AGENTS AND FLUID PRIMING IN HEMORRHAGIC SHOCK

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A. *Alkalinizing therapy in hemorrhagic shock.* Attention has been recently redirected to the rôle which acidosis plays in the development of hemorrhagic shock. In fact, Arimoto et al. have recently inculcated the plasma CO_2 capacity values, following a moderate thirty minute hemorrhagic period, into a schema for determining the probability of survival following subsequent hemorrhages (1). Prior to this in a series of hemorrhagic shock studies, Levine and co-workers stressed the importance of developing acidosis and obtained remarkable recoveries in comparison with 75 per cent mortality under control conditions by the expedient of supplementing withdrawn blood reinfusion with intravenous administrations of $NaHCO_3$ plus a metabolizable substrate, glucose (2). They intimated that many conditions which have been referred to as *irreversible* shock can probably be treated successfully in the same way.

The authors recently reported that 77 per cent mortality is to be expected when animals are subjected to hemorrhagic hypotension of 40–45 mm. Hg for 90 minutes following which the original blood volume is restored (3). It was pointed out that despite the apparently satisfactory condition of such animals for variable periods after the reinfusion of withdrawn blood, certain physiological manifestations could be detected which indicated the existence of a condition incompatible with survival (3, 4). It was postulated that once this *irreversible* condition has been established, none of the known remedial measures can affect complete recovery. The apparent similarity, however, between the intensity of the hemorrhagic condition produced by Levine's and our procedures (as judged by the essentially equivalent mortality rate in control experiments) raised the issue as to whether the condition we referred to as *irreversible* might be affected favorably by correction of the acidosis at the time of reinfusion. In an attempt to decide this issue as well as to gain further information concerning the value of alkalinizing treatments in severe hemorrhagic conditions, the following study was undertaken.

Procedure. The same number of animals (13) which were employed in the control series were subjected to the operative and standardized hemorrhagic-hypotension procedures described elsewhere (3, 5). Novocaine anesthesia was administered at the operative sites for blood vessel cannulation. All blood was let into Liquaemin[1] solution. As an extra precaution, it was filtered through cotton gauze prior to its reinfusion into the animal at body temperature. The two most satisfactory alkalinizing agents in Levine's report were selected for

[1] We are indebted to Roche-Organon, Inc., Nutley, New Jersey, for their generous supply of Liquaemin solution (anticoagulant).

trial at equivalent dosages. Seven dogs received $NaHCO_3$ (0.43 gram/kgm.) and *6* received sodium lactate (0.58 gram/kgm.) by vein in 50 to 75 ml. of water. As in Levine's experiments, a metabolizable substrate (glucose, 0.38 gram/kgm.) was added directly to the blood returned to each animal. Thus, equimolar quantities of Na^+ and glucose were introduced to the various dogs. The bicarbonate solution was administered slowly and separately to avoid unnecessary hemolysis. Within the hour following reinfusion of blood and other substances, the cannulae were removed and the wounds sprinkled with sulfanilamide powder and sutured. The animals were thereupon returned to their quarters where they were permitted to drink small quantities of water. The following day, survival animals were offered their regular quotas of food and water. No infections were seen at any time.

In *5* dogs plasma CO_2 capacity values were determined by the Van Slyke manometric method. Blood samples for the latter were drawn *a*, during the control period, and *b*, at the termination of the 90 minute hypotension period immediately prior to the intravenous injection of withdrawn blood and supplemental alkalinizing agents. Values obtained from the latter samples are designated as *terminal-hypotensive* plasma CO_2 capacities. These were desired so that the comparative degree of acidosis induced in Levine's and these animals might be evaluated.

TABLE 1

Plasma CO_2 capacity in volumes per cent

EXPT. NO.	CONTROL VALUE	TERMINAL-HYPOTENSIVE VALUE	ACTUAL REDUCTION IN PLASMA CO_2 CAPACITY
53	46.76	24.02	−22.74
54*	45.50	23.67	−21.84
55*	44.28	15.64	−28.64
56	52.05	18.70	−33.35
57	56.68	17.25	−39.43

* Indicates complete recovery animals.

Results. The occurrence of a low (about 15 vols. per cent) plasma CO_2 capacity was considered by Levine et al. to indicate the existence of a hemorrhagic condition comparable to the so-called irreversible state of others. Hence, it was taken by them as an indication for the institution of reinfusion measures. Their *terminal-hypotensive* plasma CO_2 values ran consistently lower than the respective values obtained in the *5* dogs studied in this series (table 1), only one of which fell as low as 15.6 vols. per cent. Though its significance is conjectural, however, the actual reduction in CO_2 capacity during the hypotension period was definitely less in Levine's animals (average reduction; Levine, −21.9; authors, −29.2 vols. per cent). This discrepancy may be partially accounted for in the abnormally low control values reported by Levine. According to the common assumption that the plasma CO_2 capacity expresses the degree of existing acidosis, it is probable that Levine's hemorrhagic proce-

dures induce a more severe acidotic condition. It will be seen, however, that it is inadvisable to construe that other conditions were as severely deranged as in our hemorrhaged animals.

The reinfusion of blood alone in control experiments led to a 75 per cent mortality in Levine's animals as compared with 77 per cent mortality in ours (fig. 1A). This might create the misleading impression that the two-shock producing procedures are equally severe. In their animals, however, $NaHCO_3$ supplements reduced the mortality rate to 38 per cent; in a smaller series of *8* dogs

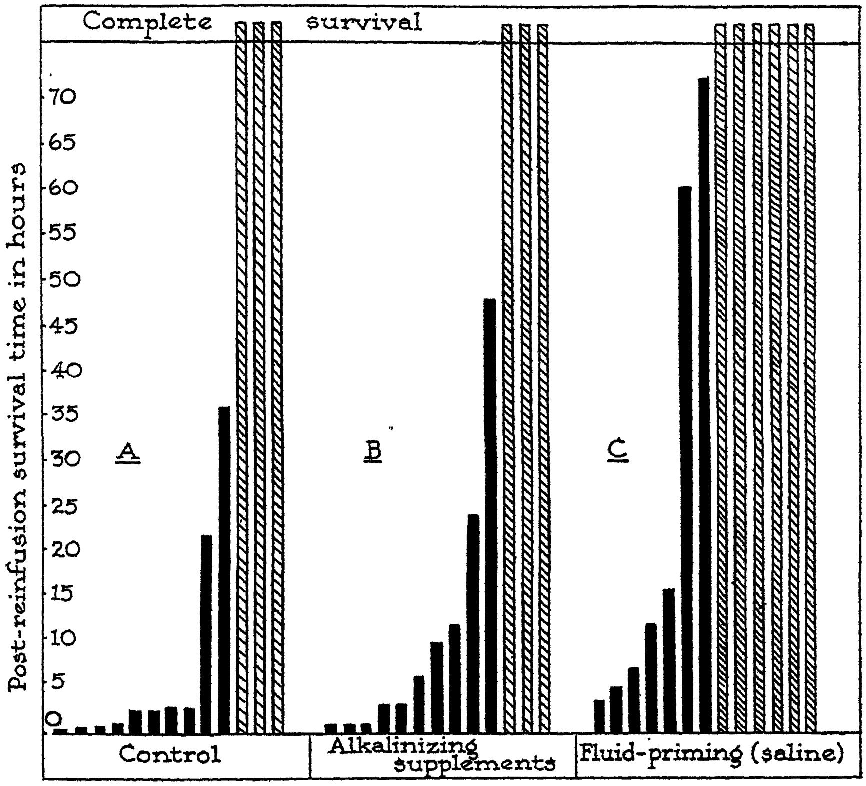

Fig. 1. Individual hemorrhagic-hypotension shock experiments

with sodium lactate supplements, the mortality rate was further reduced to 25 per cent. In contrast, the animals in this study which received one of these alkalinizing agents were not permanently benefited; that is, the mortality rate was the same as that obtained in the control series (fig. 1A and 1B).

Though no increase in the number of complete recoveries could be demonstrated, there was evidence to suggest that these anti-acidotic measures might prove valuable *if* administered during the *impending* shock state while conditions are progressing toward *irreversibility*. Comparing figure 1A and 1B, for instance, it is seen that the length of the post-reinfusion survival time is generally

longer in the latter series. There was also one less "precipitant death"[2] in the B group. Additional evidence is drawn from a comparison of the post-reinfusion (P–R) arterial blood pressures in the control, the alkalinizing and the fluid priming series (table 2). The more satisfactory pressures seen in the alkalinized group undoubtedly reflects the beneficial effects of these agents on vasomotor and cardiac function, either directly and/or indirectly through correction of the existing acidosis. It must be again re-emphasized that such excellent arterial pressures, which are only temporarily sustained in the true shock animals, tend to mask the existence of a critical state and hence, must not be permitted to influence attempts at prognosis in this period.

Discussion. On the basis of many hemorrhagic-hypotension experiments, it has been suggested (4) that, following a severe hemorrhage, an animal may progress from *a*, a state of simple hemorrhagic-hypotension to *b*, an *impending shock* state, which, if unsuccessfully checked by suitable treatment, transcends into an *irreversible shock* state, from which recovery, by definition, is impossible. Several criteria by means of which the occurrence of the transition from the

TABLE 2

SERIES	NUMBER OF ANIMALS	AVERAGE ARTERIAL PRESSURES IN MM. Hg		
		Control	P-R*	Range of P-R values
I Control	13	141	93	40–115 (170†)
II Alkalinizing supplements	13	140	132	110–150
III Fluid priming	13	140	116	70–140

* P-R refers to pressures taken within 5 minutes after reinfusion of withdrawn blood.
† This exceptional value occurred in a complete recovery dog in which the control value was also 170 mm. Hg. It was far out of line with the other values in this group.

impending to the *irreversible* shock state can be recognized have been presented (4). The findings with alkalinizing supplements in the present study support the above considerations. It was evident that once a recognizable *irreversible* state had developed in these animals, neither the return of all withdrawn blood nor the supplemental rapid correction of the existing acidosis exerted other than temporarily beneficial effects. The criteria by means of which transition from the *impending* to the *irreversible* shock state can be diagnosed were absent in the *3* complete recovery dogs in the alkalinizing series. In fact, their performance throughout the hypotension period mirrored that of the *3* survival animals in the control series. The authors feel it is safe to assume that the former would have recovered had blood alone been returned to them without the alkalinizing supplements.

It is interesting to compare these negative anti-acidotic influences upon mortality rate with the promising effects attained by Levine, especially since the mortality rate in both control series seemed to portend the production of hemor-

[2] Animals which succumbed within one hour following reinfusion of withdrawn blood were classified as "precipitant deaths".

rhagic conditions of essentially equivalent severity. Perhaps the apparent discrepancies can be quite well reconciled by the following considerations. The average mean blood pressure sustained during the hemorrhagic period was considerably higher in Levine's animals. This indicates that in general tissue blood flow was more satisfactory and the resultant degree of tissue anoxia was less severe. The greater duration of the hemorrhagic-hypotension period, however, probably accounts for the more severe terminal acidosis attained in their dogs. Since more time was available for the developing acidosis to exert its deleterious effects, it is possible that the acidotic condition was a major factor in the development of shock in their experiments.

On further analysis, it appears that Levine's hemorrhagic procedures usually lead to the development of a shock condition which may be considered irreversible in the limited sense that restoration of the original blood volume cannot effect complete recovery. In such instances, unlike the situation in this study, the transition from the *impending* to the *irreversible* shock state must occur sometime during the post-reinfusion period. Presumably, this may be attributed to the continuance of the severe acidotic condition developed during the hypotension period, since remarkable recoveries were attained by correcting this condition at the time of reinfusion. One would infer, therefore, that the successful treatment with alkalinizing substances depends upon their introduction during the *impending shock* state regardless of whether it happens to be during the hypotension or post-reinfusion period. Likewise, it follows that attempts were made in this study to treat an irreversible condition, inasmuch as alkalinizing agents were unable to alter the mortality rate.

On the basis of Levine's promising reports, as well as on the temporary benefits gained through alkalinization in our dogs, the value of such measures in severe hemorrhagic conditions warrants further investigation. Though acidosis appears to assume considerable importance in the production of hemorrhagic shock, the participation of other factors is suggested by the fact that our dogs developed *irreversible* shock with plasma CO_2 capacities considerably higher than those reported by Levine for animals, which by comparative standards were still in the *impending* shock state.

B. *Fluid priming—an anti-hemorrhagic shock measure.* Though only temporary benefits may be gained by specialized treatments when the *irreversible* state has been established, there is ample evidence that the development of the irreversible condition may be prevented by the institution of measures other than blood or plasma transfusions either prior to the loss of blood or during the *impending* shock state. The experimental reports of Ebert (6), Weston (7), and Ivy (8) strongly suggest that the resistance of an animal to the effects of severe hemorrhages may be related to the state of the animal's hydration. It has been our experience that animals which recover completely from the standardized hemorrhagic procedures or whose postreinfusion survival periods were greater than 10–12 hours revealed a common tendency to undergo continuous hemodilution for 60 to 90 minutes of the hypotension period. In those which succumbed in less than 10–12 hours, however, hemodilution processes were

arrested early with relative hemoconcentration usually setting in. On the assumption that the ability of these animals to hemodilute is an expression of their initial state of hydration, it seemed that the mortality rate following the above-mentioned hemorrhagic-hypotension procedures might be considerably reduced if the initial state of hydration before bleeding were improved.

Procedure. The method chosen to insure a well hydrated state involved sizeable intravenous injections of physiological saline at an adequate interval prior to the initiation of bleeding procedures. Each animal received, via a femoral vein, 35 ml. of saline per kilogram of body weight (about 5 per cent of the body weight) which was previously warmed to body temperature. After allowing 30–45 minutes for adequate distribution of this fluid among the various body water compartments, bleeding was begun and the remainder of the experiment was conducted according to the routine procedures previously outlined (3). Blood and plasma specific gravity determinations were made from blood samples taken at regular, arbitrarily selected intervals. The Barbour and Hamilton method for determining specific gravity was employed (9).

Results and discussion. These fluid primed animals definitely surpassed those of the control and alkalinized animals in their ability to withstand hemorrhagic-hypotension procedures of equivalent severity. The beneficial effects of priming are most clearly established in the greater number of complete recovery animals (compare fig. 1 A, B and C). Six complete recoveries are to be compared with 3 in each of the other series. In addition, it will be noted that there were no "precipitant deaths" among the non-survival dogs (fig. 1C). In fact, the dog which succumbed in the shortest period of time (3.7 hrs.) was suffering a severe attack of distemper, which, unfortunately, was not recognized until the experiment was well advanced. In the other extreme, *2* survived more than 60 hours after reinfusion. There was considerable question as to whether these could be considered as shock deaths. The definite diagnostic criteria of irreversible shock (4) were not detected in these *2* dogs. Furthermore, the intestinal congestion seen at autopsy was extremely mild. For want of more positive evidence to the contrary, however, as well as to avoid excessive enthusiasm for the effects of fluid priming, these two animals are arbitrarily designated as *delayed shock* cases.

Further evidence of the greater compensatory powers of the fluid primed dogs is presented in the following considerations. It was observed that a greater volume of bleeding was required to maintain these fluid primed dogs at the stipulated hypotensive level throughout the 90 minute period. The average total hemorrhage in the *26* dogs of the control and alkalinizing series amounted to 4.47 per cent of the body weight, whereas in the primed series it reached 5 per cent. There was also a persistent tendency for blood pressure to ascend above the established 40–45 mm. Hg level in the latter dogs. Such results are not surprising, since these animals have a greater than normal fluid volume from which to draw upon in making readjustments of the circulating blood volume to changing conditions. Depending upon the intensity and duration of any hemorrhagic-hypotension procedure, there is apparently a variable though

critical *irreducible minimum* to which cellular and osmotically active constituents of the blood can be reduced if, upon their restoration, complete recovery is to be expected. It might be surmised that this *minimum* is either lowered or less easily attained in the primed animals which are more adequately prepared to sustain a reasonably efficient circulating blood volume by auto-transfusion.

Since the total amount of blood withdrawn under these routine procedures seems at least partially to express the capacity for auto-transfusion, further analysis of the bleeding volumes seems warranted. In holding to the specifications of the hemorrhagic hypotension method, it was necessary to bleed an average of 5 per cent of the body weight in *a*, the complete recovery animals, and *b*, in those whose post-reinfusion survival time exceeded 12 hours. In a further breakdown, it is revealed that the average hemorrhage among all *non-survivals* was 4.3 per cent as contrasted with 5 per cent of the body weight in the *long survival* group. If the latter group is excluded the average total hemorrhage for those which succumbed within 12 hours drops to 4 per cent.

It appears, therefore, that the insurance of adequate hydration by saline administration before or during hemorrhagic conditions will not necessarily prevent the development of *irreversible* shock. There is little question, however, that it may prove useful in delaying the onset of irreversible conditions until more effective agents such as blood or plasma are rendered available. The advisability of beginning hydration procedures as soon as possible in severe hemorrhagic conditions is obvious from the results of the above findings in primed animals.

SUMMARY AND CONCLUSIONS

With the revival of interest in the contribution of acidosis as a primary factor in the development of hemorrhagic shock, it was decided to test the value of correcting the acidosis developed after 90 minutes of hypotension by means of sodium bicarbonate and sodium lactate. It was observed that neither of these agents, when introduced intravenously as supplements to the reinfusion of all previously withdrawn blood at the end of the 90 minute hypotension period (40–45 mm. Hg) had any effect whatsoever on the mortality rate established in a control series. These results support the authors' contention that, despite the apparently favorable behaviour of an animal, no known remedial measure can exert other than temporarily beneficial effects when administered after an *irreversible* state, as recognized by definite criteria, has developed.

Evidence of a circumstantial nature was cited to demonstrate that the administration of alkalinizing agents during the *impending* shock state may prevent or delay the transition to the *irreversible* state.

The failure of many animals to show continuous hemodilution for at least the major portion of the 90 minute hemorrhagic-hypotension period led us to believe that this process may have been restricted by an inadequate initial state of hydration. It was found that this compensatory mechanism was improved in many animals which had received (before hemorrhage) an intravenous injection of saline equivalent to 5 per cent of their body weight. The striking re-

duction in the mortality rate when compared with control animals (55 vs. 75 per cent mortality respectively) in addition to the elimination of "precipitant deaths" and the general prolongation of the post-reinfusion survival times offer strong testimony for the vital role which this compensatory mechanism plays in combatting the stress of severe hemorrhages. It also re-emphasized the importance of maintaining an adequate state of hydration whenever the loss of blood is significant.

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AN EXPERIMENTAL ANALYSIS, BY MEANS OF ACETYLCHOLINE HYPOTENSION, OF THE PROBLEM OF VAGAL CARDIO-ACCELERATOR FIBERS[1, 2]

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The earlier evidence in favor of the existence of cardio-accelerator fibers in the vagus nerves was based on the results of experiments on atropinized dogs in which stimulation of the peripheral end of the cut vago-sympathetic trunk was found to produce acceleration of the heart (13–15). In other experiments (1, 2, 6, 12) the results could not be interpreted in favor of the presence of such fibers. Recently Kabat (11) has reviewed the literature on the subject and has presented results which indicate that acceleration of the heart does occur in the atropinized dog in response to electrical stimulation of the peripheral end of either cut vagus.

Marked acceleration of the heart in response to stimulation of the vagal rootlets intracranially both before and after atropinization was reported by Jourdan and Nowak (9, 10). Repetition of these experiments by Kabat (11) resulted in moderate acceleration in only three of six atropinized animals and in negative results, i.e., slowing in eight animals without atropine.

Additional evidence in favor of vagal cardio-accelerator fibers has been obtained from experiments on the effects of acute cerebral anemia in the isolated cross-circulated head connected to its atropinized trunk only by the vagus nerves (Jourdan and Nowak,10; Kabat, 11).

In completely sympathectomized dogs (3) and cats (4, 7) exercise has been found to produce a greater increase in heart rate than that which results from section of both vagus nerves. The interpretation of this finding is difficult without the employment of vagal cardio-accelerator fibers.

Attempts to reflexly accelerate the heart through vagal cardio-accelerator fibers have led to conflicting results (Jourdan and Morin, 8; Kabat, 11).

It is evident that most of the available evidence concerning the question of cardio-accelerator fibers in the vagus is based on acute experiments in anesthetized animals and that the type of stimulus used cannot be considered physiological. Many anesthetic agents are known to depress or otherwise modify visceral reflexes to the extent that data obtained from experiments in which they have been used are unreliable. Accordingly it has seemed desirable to study the problem from the standpoint of the ability of such a physiological stimulus as a lowered blood pressure reflexly to accelerate the heart in unanesthetized trained animals so prepared that the acceleration observed may be attributed to vagal cardio-accelerator fibers.

[1] Aided by grants from The John and Mary R. Markle Foundation and the General Research Council of the State of Oregon System of Higher Education.

[2] A preliminary report has been published (5).

METHOD: The sharp profound fall of blood pressure needed for the purpose of reflexly activating cardio-accelerator mechanisms was produced accordiing to a method described by Youmans, Aumann, Haney and Wynia (16) in which the depressor compound acetylcholine was administered intravenously in selected doses. In all of the present experiments the doses varied from 0.5 to 1.5 mgm. of the compound. Variations in response could not be correlated with the dose selected.

In some of the experiments intravenous injection of nitroglycerine in doses of 1 or 2 cc. of 1 to 2500 dilution was used as a means of producing the fall of blood pressure.

All surgical procedures were carried out under nembutal anesthesia. Sympathetic denervation of the heart was accomplished by bilateral removal of the stellate and upper five or more thoracic sympathetic ganglia. Animals having had this operation will be spoken of as cardiac sympathectomized or as dogs having sympathectomized hearts. The vagi were sectioned at about the level of the thyroid cartilage.

The adrenal demedullation consisted of a thorough cauterization of the entire core of the glands. Later histological examination of some of the glands revealed not more than traces of medullary tissue.

All results were obtained from experiments on chronic dog preparations without the use of anesthesia. For purposes of injection a sharp 22 gauge needle attached to a syringe containing 0.9 per cent NaCl solution was carefully introduced into a leg vein. The trained animal exhibited no evidence of excitement. Following an interval sufficient to determine that the heart rate was reasonably well stabilized, the continuous electrocardiographic tracing was started. After about 20 seconds of recording, the acetylcholine was injected rapidly from another syringe attached through a three-way valve with the same needle. The electrocardiogram was continued for 60 to 80 seconds or longer after the injection. The record obtained was divided into five-second intervals before and after injection and the corresponding heart rates determined.

RESULTS. 1. *Normal animals.* In 23 experiments on 9 normal dogs before injection of acetylcholine the average heart rate was 105 beats per minute and the range was from 78 to 136. The maximal acceleration occurred 10 to 15 seconds following injection and the average rate reached a maximum of 251 beats per minute at 25 seconds. The individual post-injection maximal rates ranged from 171 per cent to 392 per cent of the corresponding pre-injection levels, the average being 240 per cent. Maximal rates in all but four experiments were reached during either the fourth or fifth five-second interval after injection, two were reached during the third, and one each during the sixth and seventh. The results of these experiments are illustrated in figure 1. The data of this group of normals were presented previously in another study (Youmans, Aumann, Haney and Wynia, 16).

2. *Sympathectomized heart.* In 21 experiments on eight dogs the average heart rate increased from a pre-injection level of 83 beats per minute through a maximal acceleration at 15 to 20 seconds to a maximum of 171 at 30 seconds. The

individual maximal rates ranged from 131 per cent to 324 per cent of the corresponding pre-injection levels, the average being 221 per cent. The data of these experiments are summarized in figure 1.

3. *Sympathectomized heart, adrenals demedullated.* In 11 experiments on four dogs the average heart rate increased from a pre-injection value of 88 beats per minute through a maximal acceleration at 15 to 20 seconds to a maximum of 181 at 25 seconds after injection. The individual maximal rates ranged from 162 per cent to 296 per cent of the corresponding pre-injection levels, the average being 218 per cent. These data are included in figure 1. In five additional experiments on four dogs in which nitroglycerine was used as the depressor agent there resulted a rise of average heart rate from a pre-injection level of 81 beats per minute through a maximal acceleration at 15 to 20 seconds to a maximum of 137 at 25 seconds after injection.

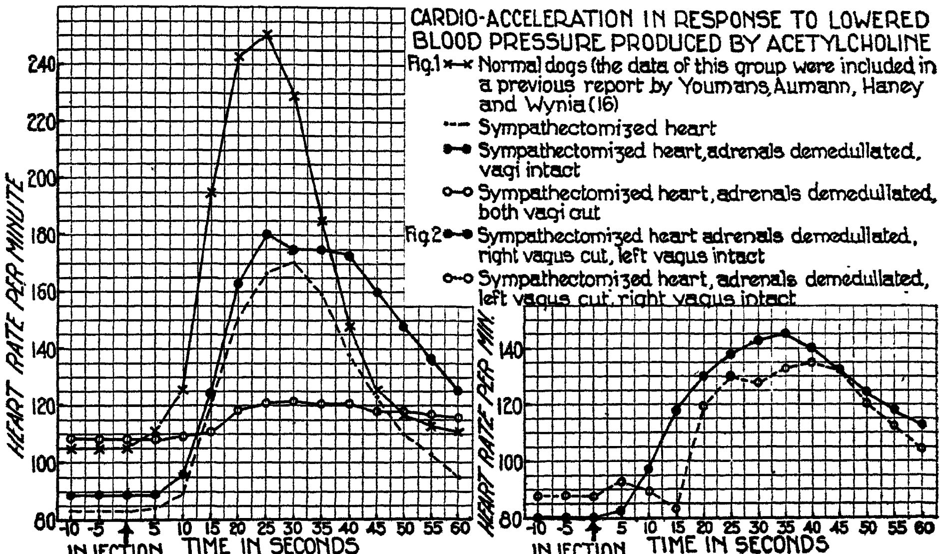

Fig. 1 Fig. 2

4. *Sympathectomized heart, adrenals demedullated, right vagus cut, left vagus intact.* In eight experiments on three dogs the average pre-injection heart rate was 80 per minute. After injection of acetylcholine the average rate rose through a maximal acceleration at 10 to 15 seconds to a maximum of 145 per minute at 35 seconds after injection. The individual maximal rates ranged from 135 per cent to 195 per cent of the corresponding pre-injection levels, the average being 167 per cent. Data on this series of experiments are included in figure 2.

5. *Sympathectomized heart, adrenals demedullated, left vagus cut, right vagus intact.* In 14 experiments using acetylcholine on four dogs the average heart rate rose from a pre-injection level of 88 per minute through a maximal acceleration at 15 to 20 seconds to a maximum of 135 per minute at 40 seconds after injection of the compound. The individual maximal rates ranged from 126 per cent to 238

per cent of the corresponding pre-injection levels, the average being 169 per cent. The data are to be found in figure 2. In four additional experiments on four dogs in which nitroglycerine was used as the depressor agent, the average rate rose from a pre-injection level of 85 beats per minute through a maximal acceleration at 10 to 20 seconds to a maximum of 121 at 30 seconds.

6. *Sympathectomized heart, adrenals demedullated, both vagi cut.* In 21 experiments on 7 dogs using acetylcholine as the depressor agent the average heart rate rose from a pre-injection level of 108 per minute through a maximal acceleration at 15 to 20 seconds to a maximum of 122 per minute at 30 seconds after injection. The individual maximal rates ranged from 110 per cent to 142 per cent of the corresponding pre-injection levels, the average being 120 per cent. The data are included in figure 1. In eight additional experiments on four dogs in which nitroglycerine was used as the depressor agent, the average heart rate rose from a pre-injection level of 111 per minute through a maximal acceleration at 20 to 25 seconds to a maximum of 118 at 35 seconds after injection.

It is interesting to note that in agreement with Youmans et al. (16), the acetylcholine apparently affects ventricular rate more than auricular. Frequently it was observed that during the second to fourth five-second intervals partial or complete A-V block developed, resulting at times in ventricular asystole for several seconds. In such cases the auricular rate was recorded in the data.

Discussion: The injection of acetylcholine in amounts sufficient to evoke a marked but brief cardiac depression results in a profound, short-lasting fall of blood pressure. The duration and extent of this hypotension are sufficient to evoke reflex activation of those mechanisms which normally operate to compensate for lowered blood pressure, (16). Experiments in which nitroglycerine was used as the depressor agent have yielded results qualitatively indistinguishable from those obtained with acetylcholine. The degree of acceleration provoked by nitroglycerine was somewhat less than that which occurred in response to acetylcholine. This difference is readily explained by the fact that the action of acetylcholine is through direct inhibition of the heart followed by a sharp, profound fall of blood pressure, while the vasodilator action of nitroglycerine causes a more gradual development of hypotension. Therefore the reflexes which act to compensate for hypotension are activated more intensively in the case of acetylcholine than in the case of nitroglycerine. Since the rise of heart rate resulting from the administration of either of these compounds is almost certainly due to the reflexes set up to compensate for hypotension, either of them may be used in attempts to activate the vagal cardio-accelerator mechanism.

In the normal animal, as would be expected, a marked cardiac acceleration follows the brief period of inhibition provoked by the acetylcholine. The maximal increases in rate ranged from 90 to 304 beats per minute.

In the dogs whose stellate ganglion and upper five or more thoracic sympathetic ganglia have been removed bilaterally, marked acceleration still occurs, the maximal increases in rate ranging from 32 to 166 beats per minute. The following possible causes of this acceleration must be considered. First it is possible that not all sympathetic fibers to the heart have been removed and that the rise

in heart rate is produced reflexly through those remaining. The fact that cardiac acceleration in response to acetylcholine hypotension is markedly reduced after sympathectomy suggests that the effect of the cardiac sympathetics has been largely if not entirely abolished. All dogs included were tested for response to acetylcholine hypotension after adrenal demedullation and denervation of the heart were completed. Only those which failed to show a marked accelerator response were included in this data.

Secondly, the adrenal medullae may respond reflexly with increased output of adrenalin. Bilateral demedullation of the adrenals of a dog having a sympathectomized heart and intact vagi did not decrease the accelerator response to acetylcholine hypotension as demonstrated in figure 1.

Thirdly, it is probable that sympathin liberated at the remaining sympathetic nerve endings circulates to the heart and provokes acceleration. Evidence in favor of the operation of this mechanism in response to acetylcholine hypotension has been given by Youmans, Haney, Lindgren and Karstens (17). The cardiac acceleration which occurs in response to hypotension in the adrenal demedullated, denervated heart animals of part 6 of the results may be attributed reasonably to sympathin. As noted in figure 1 the increase of average rate was only 14 beats per minute.

Fourthly, reflex inhibition of tonically active vagal cardio-inhibitory fibers might account for at least a part of the acceleration of the heart which occurs in response to hypotension in the dog having a sympathectomized heart. It will be noted in part 3 of the Results that in dogs having sympathectomized hearts and demedullated adrenals a rise of 93 beats per minute in average heart rate occurred following the injection of the depressor agent. The question arises as to how much of this increase may be attributed to reflex inhibition of the cardio-inhibitory fibers of the vagus. This would seem to be answered by the data of part 6 of the results illustrated in figure 1 in which the average pre-injection heart rate after bilateral vagotomy is only 108 beats per minute, i.e., only 20 beats above the resting pre-injection rate shown in the data of part 3. It would seem that reflex depression of the vagal cardio-inhibitory fibers could not account for more acceleration than that which results from section of the nerves, and that it may be used to account for only a part of the rise of heart rate which occurs in response to hypotension in the animals of part 3 of the results.

Fifthly, the rôle of non-sympathetic vagal cardio-accelerator fibers must be considered as a possible explanation for at least a part of the cardiac acceleration which occurs in response to the hypotension produced by acetylcholine or nitroglycerine. In order to test this possibility, as indicated in the results, dogs were prepared as follows. The adrenals were demedullated bilaterally. Following recovery from the second demedullation the stellate and upper five or more thoracic sympathetic ganglia were removed first on one side and later on the other. In such a chronic animal preparation there are three possible explanations for the cardiac acceleration which occurs in response to hypotension. The first is concerned with reflex depression of the vagal cardio-inhibitory fibers, the second with reflexly liberated sympathin and the third with possible accelerator fibers

in the vagus nerves. In this animal having no adrenal medullae and a sympathectomized heart, marked acceleration still occurs in response to hypotension produced by acetylcholine or nitroglycerine, the average heart rate in response to the former showing a rise of 93 beats per minute. If the vagi now are sectioned, the resting heart rate is elevated presumably as a result of the removal of the tonic inhibitory influence of the vagi on the heart. Thus the average rate in the animals of part 3 is 88 per minute and that of part 6 is 108 per minute, a difference of only 20 beats per minute as shown in figure 1. This elevated rate after vagotomy is much lower however than that reached in response to hypotension before the vagi were cut. Therefore inhibition of vagal cardio-inhibitory tone can explain only a part of the acceleration which occurs in response to acetylcholine hypotension. If one adds the 14 beats per minute attributable to sympathin in the animals of part 6 of the results to the 20 beats attributable to reflex removal of vagal cardio-inhibitory tone, the result is the maximum increase which can be attributed to these two, i.e., 34 beats per minute. Yet the adrenal demedullated, sympathectomized heart animals with both vagi intact show a rise of 93 beats per minute. It seems probable that the difference of 59 beats per minute may be attributed to vagal cardio-accelerator fibers.

Following bilateral vagotomy of the adrenal demedullated, cardiac sympathectomized dog, the heart still is accelerated in response to hypotension produced by acetylcholine or nitroglycerine, the maximal rise of average rate being only 14 beats per minute as indicated in part 6 of results and in figure 1. This acceleration however is much lower than that which occurs in response to the same doses of the depressor agents before vagotomy. As far as sympathin is concerned, it should not enter into the picture differently before than after vagotomy unless it may be assumed that following a dose of the depressor agent the blood pressure remains low for a longer period of time in the animals after vagotomy than before. This possibility is incident to the removal by vagotomy of cardio-accelerator fibers and of the factor of reflex depression of vagal inhibitory tone. Such a possible difference in the rôle of sympathin obviously does not help to explain the fact that there is a greater accelerator response to hypotension before than after vagotomy. It seems certain then that reflex liberation of sympathin fails to account for the cardiac acceleration which occurs in these experiments.

Neither reflex depression of vagal cardio-inhibitory tone nor reflex liberation of sympathin will explain the major part of the increase of heart rate which occurs in response to acetylcholine or nitroglycerine hypotension in the adrenal demedullated, cardiac sympathectomized animal. It is clear that vagal cardio-accelerator fibers could readily account for the greater part of the acceleration.

It seems reasonable to expect that the terminal distribution of cardio-accelerator fibers in the vagus nerves might correspond with that of the inhibitory fibers, i.e., those of the right vagus predominantly to the S-A node and those of the left to the A-V nodal region. The experiments of Kabat (11) in which accelerator effects were obtained principally from the right vagus led to the conclusion that the vagal cardio-accelerator fibers are located predominantly in the right vagus. As indicated by the data presented in figure 2, our results lend no support to this

conclusion. The rise of average heart rate in adrenal demedullated, cardiac sympathectomized animals having the left vagus intact was 65 beats per minute while in those having the right vagus it was 47.

A knowledge of the degree to which vagal cardio-accelerator fibers are responsible for the acceleration of the heart which occurs in response to hypotension in the normal animal is desirable. It would seem that a comparison of the degree of acceleration in response to hypotension in the adrenal demedullated cardiac sympathectomized animal with that which occurs in the adrenal demedullated vagotomized animal having intact sympathetics might be of aid in settling the point. This comparison, however, is complicated by the fact that in the former animal both reflex inhibition of vagal cardio-inhibitory tone and reflex liberation of sympathin at extracardiac sympathetic nerve endings as well as the vagal cardio-accelerators must be considered. In the latter animal the comparison also is complicated by the fact that consideration must be given to reflexly liberated sympathin from extra-cardiac sympathetic nerve endings as well as to the direct effect of cardiac sympathetics.

Thus it would appear that any generalization regarding the relative importance of vagal cardio-accelerator fibers must be based on the facts that the adrenal demedullated cardiac sympathectomized animal responds to acetylcholine or nitroglycerine hypotension with a much greater acceleration of the heart before than after vagotomy, and that neither reflex depression of the vagal cardio-inhibitory tone nor reflexly liberated sympathin accounts for all of this difference in response.

The possibility must be considered that acetylcholine may directly stimulate sympathetic ganglion cells which were not destroyed by the cardiac sympathectomy. Such ganglion cells might possibly be located in cervical ganglia. Their axones could reach the heart through the vagal trunks. If this mechanism were to explain the part of the acceleration not accounted for by depression of vagal cardio-inhibitory tone and sympathin, it follows that the acceleration provoked by nitroglycerine should not be greater than that so accounted for. Nitroglycerine administration is followed by a rise of 56 beats per minute in the animals of part 3 of the results, and by a rise of 7 beats per minute in those of part 6. Thus the difference in response before and after vagotomy is 49 beats per minute. As noted previously, the results indicate that only 34 beats per minute are attributable to the combined influence of reflex depression of vagal inhibitory tonus and of sympathin. There remains to be accounted for 15 beats per minute. Since nitroglycerine is not known to stimulate ganglion cells, it is probable that reflex stimulation of vagal cardio-accelerator fibers accounts for a part of the increase of heart rate which follows nitroglycerine administration.

Another source of acceleration to be considered concerns a possible pharmacologic action of acetylcholine directly on the heart or upon ganglion cells in the heart. This action should not be destroyed by vagotomy and therefore could not account for the great difference in accelerator response of the animals before and after vagotomy. Further studies on the possibility of such a direct action are in progress.

The evidence presented favors the presence of vagal cardio-accelerator fibers

which are excited in response to hypotension in the unanesthetized, cardiac sympathectomized, adrenal-demedullated dog. It is reasonable to assume that such fibers play a rôle in the cardiac acceleration which occurs in response to lowered blood pressure in the normal animal.

SUMMARY

Arterial hypotension induced by either acetylcholine or nitroglycerine provokes a marked acceleration of the heart in the normal unanesthetized dog. Cardiac sympathectomy and adrenal demedullation reduce but do not abolish the response. The consideration of neither reflexly liberated sympathin nor reflex depression of vagal cardio-inhibitory tone is capable of accounting entirely for the acceleration which occurs in response to hypotension in unanesthetized, adrenal-demedullated, cardiac sympathectomized dogs. A reflex excitation of cardio-accelerator fibers of both vagus nerves readily explains the acceleration.

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THE DETERMINATION OF THE PROPAGATION VELOCITY OF THE ARTERIAL PULSE WAVE[1]

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As judged from the period of the standing wave, Moens (1) in 1878 concluded that the velocity of a pressure pulse wave through an elastic tube was related to the elastic properties of the wall of the tube and to the density of the fluid medium. By making several critical assumptions, the most significant being that the modulus of elasticity be constant, the thickness of the wall be neglibible as compared to the internal diameter of the tube, and the pressure wave generated be small, he set up the formula:

$$Vp = K\sqrt{gEa/wd} \tag{I}$$

in which Vp is the pulse wave velocity in meters per second; g, the gravitational constant; E, Young's modulus for the wall of the tube, in grams per square centimeter; a, the thickness of the wall in centimeters; w, the density of the fluid in grams per cubic centimeter; and d, the internal diameter of the tube, in centimeters. On the basis of his experiments with various elastic tubes, he gave the constant the value of 0.9.

Moens also made some attempt to apply his formula to physiological systems. He first showed that values for pulse wave velocity derived from the elastic modulus of an arterial segment were of the same order as those given in the literature for the living animal. When the actual velocity of a pulse wave through a length of intestine or aorta was compared with the derived velocity, agreement was good, although not strictly quantitative.

Meanwhile Korteweg (2) had constructed, on theoretical grounds, an equation which should predict the propagation of a wave whose speed is governed by the lateral displacement of the walls of an elastic tube, assuming any longitudinal stretch to be negligible. His formula differs from that of Moens only in the absence of the constant.

In 1922 Bramwell and Hill (3) applied the Korteweg formula, which they attributed to Moens (see 4), to their studies on the velocity of the arterial pulse. For simplicity, the density of blood was assumed to be constant at 1.055, and the thickness of the wall was neglected. They then showed that the elastic modulus could be expressed in terms of the volume and pressure change by:

$$E/d = \Delta pV/\Delta v$$

When the units of pressure are changed to mm. Hg, the Korteweg formula then becomes:

$$Vp = 0.357/\sqrt{\Delta pV/\Delta v} \tag{II}$$

[1] The expenses of this investigation were partly defrayed by a grant from the American Medical Association.

in which Δp was the increment in pressure in millimeters of Hg corresponding to Δv, the increment in volume in cubic centimeters starting from V, the initial volume of the tube.

When the formula was applied to the tension-length figures given by Roy (5) for arterial segments, the calculated pulse wave velocity was found to be constant for diastolic pressures between 20 and about 60 mm. Hg, after which the velocity increased as the pressure was increased. A similar relationship between velocity and diastolic pressure was found when artificial waves were generated in a length of human carotid artery filled with mercury.

The next year (6), with refined apparatus, they re-established the relation between the velocity of generated pressure waves in a carotid segment and the diastolic pressure. These observers had little more success than Moens in establishing quantitative accord between the observed velocities and those calculated from the tension-length curves, but since the observed and predicted curves were of the same form, they believed that the formula would predict the pulse wave velocity if corrected for the stream velocity. The viscous drag of the arterial wall when subjected to a stretch as rapid as that occurring in the body, was suggested as a factor which might cover any discrepancy which still existed between calculated and observed values.

Little (7) found satisfactory agreement between measured transmission velocities in a very long rubber tube and those calculated from the stretch characteristics of the wall. Further, Hallock and Benson (8) obtained good agreement between predicted and actual velocities of an artificial pulse wave in a length of aorta. This accord was reached, however, only when stream velocity and viscous action of the aorta were corrected for by assumed constants.

It should be recognized that physiological systems violate the fundamental assumptions on which are based the classical equations relating distensibility to pulse wave velocity. Arterial pulsations are too large, the wall too thick and the tension-length curve of vascular tissue is not linear. It is not surprising, therefore, that the correlation between pulse wave velocity as actually measured and as calculated from distensibility measurements, is, to say the least, only qualitative. Yet it is on such an insecure basis that attempts have been made (9, 10) to predict the cardiac stroke volume from the pulse pressure and a distensibility figure derived from the pulse wave velocity. We therefore decided to restudy the relation between pulse wave velocity and the elastic properties of the tube, starting with a system similar to that used by Moens, i.e., a length of Gooch tubing, and ending with an analysis of the propagation velocity of pressure pulses experimentally developed in the aorta.

I. *The velocity of pressure pulses generated in a length of Gooch tubing.* The Gooch tubing was selected to satisfy as closely as possible the assumptions of Moens. It had an internal diameter of 15.3 mm. and a wall thickness of 0.8 mm. More important, in the range of tension employed, the coefficient of elasticity was constant, so that the half-circumference of a 10 mm. length gained 0.8 mm. with every 50 grams increase in tension (fig. 1). When tension is converted to internal pressure, and half-circumference to internal volume, the pressure-

volume relationship is, of course, no longer linear (fig. 1). Since the Bramwell and Hill formula states that the wave velocity will depend inversely on relative increase in volume per unit pressure rise, it is obvious that the velocity in this rubber system should decrease as the diastolic pressure is increased (fig. 1).

To follow the transformation of a generated pulse wave as it was propagated through various lengths of Gooch tubing, at least two, and usually three, optical manometers of adequate frequency were connected to needles, placed at the ends of the tube and at various points between the ends. Fluid was injected into one end from a burette under air pressure, so that a known amount could be delivered very rapidly. A desired basal (diastolic) pressure level was established by an elevated reservoir bottle, connected into the injection system by a double stopcock. Pressure waves were also generated by means of a wooden "nut cracker," placed between the reservoir bottle and the Gooch tube, so that a length of heavy-walled rubber tubing could be rapidly compressed. By using a

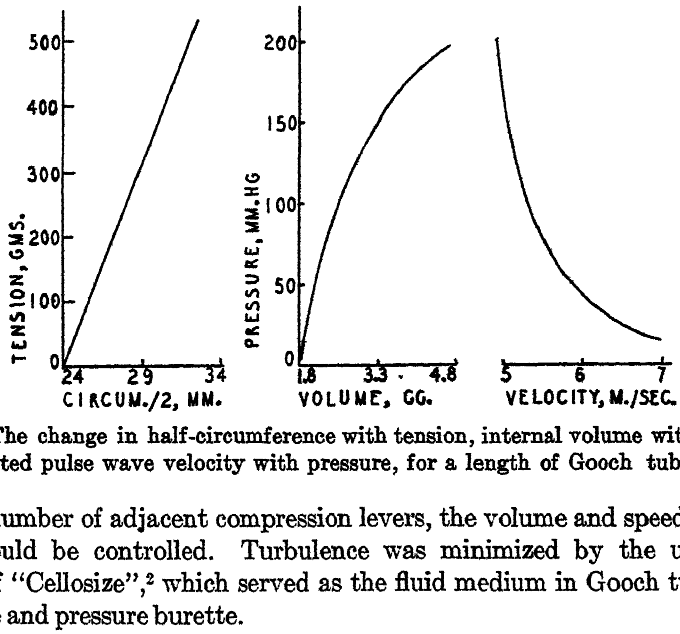

Fig. 1. The change in half-circumference with tension, internal volume with pressure, and calculated pulse wave velocity with pressure, for a length of Gooch tubing.

different number of adjacent compression levers, the volume and speed of the injection could be controlled. Turbulence was minimized by the use of 1:4 dilution of "Cellosize",[2] which served as the fluid medium in Gooch tube, reservoir bottle and pressure burette.

A graphic illustration of the transformation of a pressure wave as it is propagated through the Gooch tube was obtained by the use of a tube 150 cm. in length, with the recording sounds placed near the ends at a separation of 146 cm., and also in the center of the tube (fig. 2). A short wave was produced, one whose length was but a fraction of twice the tube length, so that the course in transmission was uncomplicated by fusion of its parts.

For example, a wave was generated at the orifice end of the tube which showed a pressure rise from an initial level of 45 mm. Hg to 75 mm. Hg (fig. 2). The

[2] We are indebted to Mr. C. A. Setterstrom of the Carbide and Carbon Chemicals Corporation for the supply of "Cellosize" used in these exeriments.

wave was 130 m.sec. in duration (from foot to the final return to initial level). Since the average propagation time of the wave was 208 m.sec., the wave length was 130/208 or 0.62 of the tube length.

The foot of the wave traversed the tube in 161 m.sec. and was therefore travelling at a velocity of 9 M. per sec. This velocity was uniform for the whole length of the tube. The peak of the pressure wave travelled at the slower rate of 7 M. per sec., which velocity was also uniform along the tube. The "tail" of the wave had still another speed (4.6 M. per sec.). The differences in velocities produced a rather rapid lengthening of the wave, so that by the time it had completed its first trip it was 1.19 × the tube length. The wave was not sym-

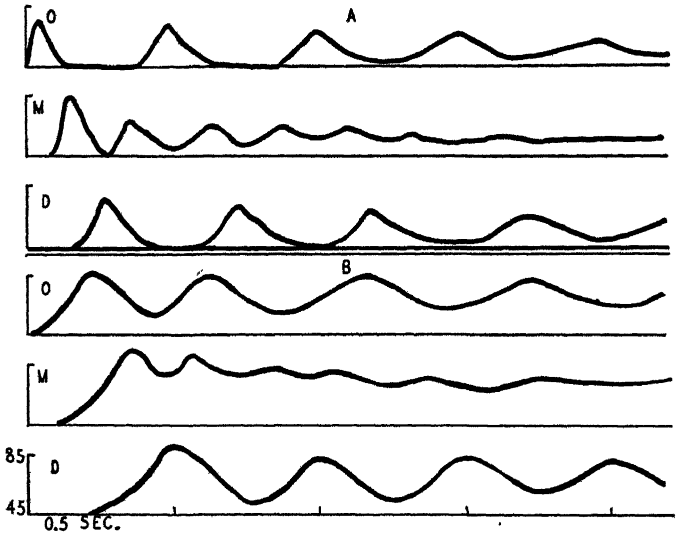

Fig. 2. The transformation of a propagated pressure wave in transit through a length of Gooch tubing. A, a short wave, less than twice the tube in length. B, a longer wave, greater than twice the tube in length. *O*, the record taken at the orifice end of the tube; *M*, taken at the mid-point of the tube; and *D*, taken at the distal end of the tube.

metrical at this time, for while the foot-to-peak phase was 0.46 of the tube length, the peak-to-tail phase was 0.73 of the tube length.

On its second trip through the tube, the foot of the wave moved at a velocity of 7.2 M.per sec., the peak at 6.5 M. per sec., and the tail at 5.0 M. per sec. At the completion of this trip, the wave length had increased to 1.40 × the tube length, with the asymmetry more pronounced. By the time of the fourth trip through the tube, the peak had reached a constant velocity of 6.2 M. per sec. The expected wave length was 2.12 × the tube length, which means that the foot was encroaching upon the tail. Symmetrical oscillations of the standing wave were not obtained until the sixth trip through the tube, when the foot had overrun the tail to the point at which the propagated wave had become symmetrical.

Several crucial facts are apparent from this analysis. First, the velocity of the

foot was always significantly greater than that of the peak of the wave. This has been substantiated by the analysis of a great number of pulses generated in various lengths of tubing. Second, the time interval between the successive arrivals of the peak of the propagated wave at the end of the tube, increased progressively until a constant value was reached, which period was then maintained until all waves had been damped out. Third, the transmission time of the foot of the wave gives no index to this constant period of the standing wave. Fourth, the propagated wave was not symmetrical as it was generated, and symmetry was attained only because the velocities of the wave parts were different so that the foot would over-run the preceding tail. Fifth, as the propagated wave became progressively longer, pressure oscillations at the middle of the tube become ever smaller, until, by the time the symmetrical standing wave has been developed, a "node" had been established with the complete elimination of pressure oscillations at the center of the tube.

Having established the fact that the wave form is changed in its transmission, the question then arises as to what factors govern the velocity with which the various parts of the wave will be propagated. The explanation offered by Bramwell (12) is that each velocity will be dependent upon the stretch characteristics of the wall obtaining at the related volume level. The velocity of the peak of a wave, for example, would be the same as if the peak were the foot of a new wave rising from the peak pressure level. Attractive as this concept is, it does not fit the data obtained in our experiments. If true, the velocity of the peak of the wave, and hence the period of the standing wave, should be influenced by the pulse pressure developed. Actually it was found that neither the velocity of the foot of a wave nor the period of the standing wave is significantly altered by changing the size of the pressure wave generated, and both are referable solely to the initial pressure volume level. For instance, when a positive and a negative wave were generated separately at the same initial pressure, both the velocity of the foot and the period of the standing wave were the same. All parts of the wave train seem dependent only upon the initial pressure volume relations.

On the other hand, when a series of waves, produced in a tube 21 cm. in length, were grouped according to the speed of the initial pressure rise, a significant correlation was revealed between the rate of pressure rise and the velocity of the pulse wave foot (table 1). This statement is not contradictory to the previous one that the propagation time was independent of the size of the pressure pulse. A small wave was propagated at the same rate as a very large one if the rates of first pressure development (upstroke of curve) of the two were the same.

The period of the standing wave proved independent of the rate of pressure rise, but the interval of time elapsing before a constant period was reached was affected. The more rapid the velocity of the foot, the greater number of trips required before a constant value was reached. It seemed, then, that the velocity of the foot must be measuring a reluctance of the wall to stretch, a "viscous action" or "hysteresis," which would be changed by differences in the rate of tension development.

II. *Measurement of the tension-length characteristics of the Gooch tubing.* The tension-length figures already cited were obtained with a Scott serigraph,[3] in which tension was progressively increased by a change in angulation of a track carrying a weighted carriage. Tension was developed from 0 to 500 grams at a uniform rate in one minute. In some cases, when longer lengths of tubing were being stretched, the carriage was weighted with 1,000 grams rather than 500 grams, so that tension levels equal to at least 300 mm. Hg internal pressure could be developed. The degree of stretch was recorded, without magnification, by a pen mounted on the carriage.

When the velocity of the pulse wave was derived from the tension-length line so obtained, it was found that its value was always less than those actually observed for the transmission of the foot of the wave. The calculated velocity was, however, quite similar to the final, constant velocity reached by the peak of the wave, which also is a measure of the period of the standing wave. In the 150 cm. tube, for example, the calculated velocity was 6.3 M. per sec., as compared with the final velocity of the propagated peak of 6.2 M. per sec.

In no system was agreement exact. It was found that the Bramwell and Hill formula (II) requires a constant, just as Moens found a constant necessary, if the period of the standing wave is to be predicted from the tension-length characteristic of the wall. The values of this constant differ with different tubes, but have varied from 0.88 to 0.97. Using a value of 0.9, as did Moens, the standing wave period can be predicted by

$$Vp = 0.9 \times 0.357/\sqrt{\Delta v/V\Delta p} = 0.32/\sqrt{\Delta v/V\Delta p} \qquad \text{(III)}$$

Since it was indicated that the acceleration of the foot of the wave might be due to a hysteresis with more rapid stretch, another apparatus was designed which would allow the recording of length and tension while tension was being developed at varied and rapid rates. The ring to be stretched was hung upon two brass pins, *p*, which were supported by vertical strings from an overhead wooden support. One of these pins was connected by means of fine fishing cord to a short stub of piano wire held in a rigid brass mount. This wire served as a stiff spring, bending through a small angle when tension was applied. At its end was placed a mirror, *m*, so that the deflections of the spring could be recorded optically. (See fig. 3.)

The other supporting pin was connected, again with fishing cord, horizontally to a pendulum, *P*, constructed from a meter stick to which weights could be bolted at any desired distance from the pivot point. Hence the total tension developed and the period of the pendulum could be varied at will.

On the right hand pin was hung a thin brass shield, *s*, behind which was placed a large front-surface mirror, *M*, so that the shadow of the edge of the shield was

[3] This instrument was lent to us by Dr. Joseph Krafka of the Department of Microscopic Anatomy, for which loan we wish to express our appreciation. More details as to the structure of the apparatus and as to the stretch records it registers will be found in Dr. Krafka's article on the stretch characteristics of the aorta (11).

thrown upon the camera slit. As the ring was stretched, the movement of this shield to the right could be optically recorded. The magnification was 1.8 ×.

The pendulum was swung off plumb for the desired distance, and supported by a trigger. The cord to the pin holding the ring was then fastened without sag, and with an initial positive tension which could be varied from a very low figure to values corresponding to various "diastolic" pressures. Upon release of the trigger, the ring was subjected to periodic stretches as the pendulum swung down and returned. The speed of tension development could be varied at will from 0.1 to 5–10 grams per m.sec. When a greater speed was desired, a stiff spring was bolted to the end of the meter stick, and secured, at its other end, in a rigid clamp. The action of the spring thus shortened the period of the pendulum.

When a length of Gooch tubing was subjected to very rapid stretches, hysteresis loops were obtained, with tension increasing more rapidly in relation to stretch as the rate of tension development was increased (fig. 4). With rates

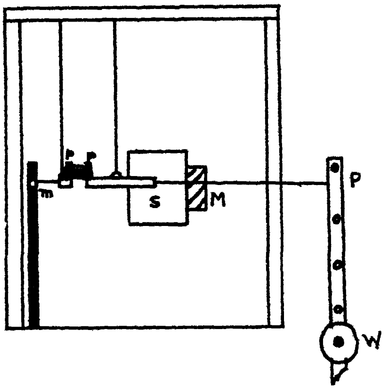

Fig. 3. Diagram of a device to register the tension and stretch of elastic material while the tension is being quickly increased. For explanation see text.

corresponding to a rise of 1.3 mm. Hg in pressure per m.sec., or less, the extensibility was significantly different from that obtained with the slow Scott serigraph only in a bowing up at the very start of the curve. As the pressure increase was made ever more rapid, this bow occupied a greater and greater part of the curve, and the extensibility over the whole range from 0 to 500 grams tension was less than with the slow tester. This hysteresis was found regardless of the basal tension level which obtained prior to the stretch. If the tension-length values for this series of curves are converted to pressure-volume, and tangents taken at the first part of the curve with a tangent meter, it is found that the greater the bowing in the tension-length curve away from the straight line characterizing a slowly developed stretch, the faster is the pulse wave velocity as calculated by equation III. In fact, velocities so derived from the first part of the rapid stretch curve show quantitative agreement with those determined experimentally for the propagation time of the foot of a pulse wave (table 1).

Curiously enough, when repeated stretches were made, with only a short rest

period between (10 to 250 m.sec.), and with the same rate of tension development with each stretch, the extensibility curves were not the same (fig. 5). With each succeeding stretch, the curve approached the straight line relationship derived from a slowly developed stretch, so that shortly the two curves were identical except for the persistence of a slight bow at the very start of the curve. It is as though the first stretch had "lubricated" the rubber, so that following stretches were more easily developed.

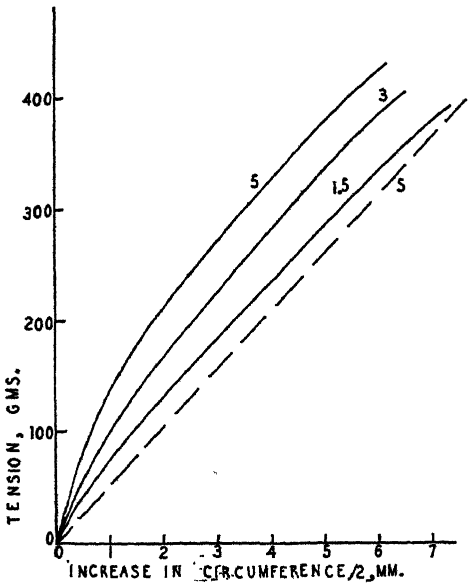

Fig. 4

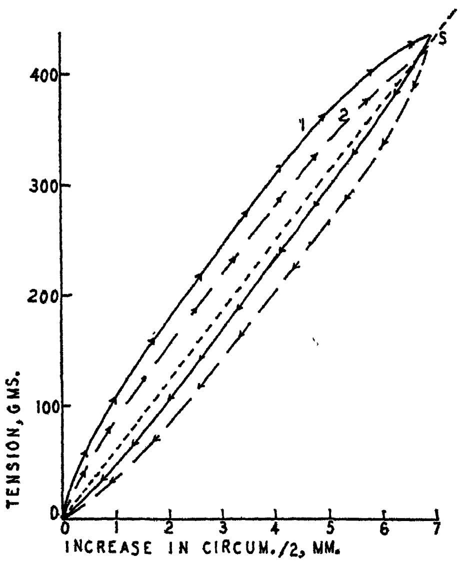

Fig. 5

Fig. 4. The influence of the rate of stretch upon the extensibility of Gooch tubing. *S*, the extensibility curve given by a slowly developed stretch (Scott serigraph); the other curves show the extensibility when tension was applied at the rate of 1.5, 3 and 5 grams/m. sec. respectively.

Fig. 5. Change in extensibility curve upon repeated rapid stretching. *S*, the extensibility curve developed by a slow stretch (Scott serigraph). *1*, the first extensibility curve; *2*, the second curve, made at the same rate of tension rise, and starting immediately upon the completion of the first cycle of stretch and return to zero tension.

It is also apparent from figure 5 that the recovery of the wall from a rapidly developed stretch does not follow the same curve taken by the extension. This recoil curve differs from the stretch curve, in that it is less vertical as it approaches initial tension than was the beginning of the stretch curve. With successive stretches, the recoil curve also shifts, first beyond the straight line of the slow stretch, and then back to it.

Velocities calculated by equation III from tangent values at the very completion of the recoil curves are quantitatively similar to those determined for the "tail" of a propagated wave. Further, the progressive shift in the stretch and recoil curves with repeated stretches will also quantitatively explain the gradual

change in the velocity of the foot and tail of a wave, as it makes repeated trips through the Gooch tube, and hence subjects the wall to repeated stretches. It seems more than coincidence that the velocity of the peak of a wave will reach a constant and predictable velocity in the same number of trips through the tube, as is required of successive stretches for a quick stretch curve to lie on the straight line slow stretch record.

In summary, it seems that the velocities of the various parts of a propagated wave are different because of a hysteresis of the tube wall with a rapidly developed stretch and rapidly occurring recoil. The more rapid the pressure rise in a generated pulse, the greater will be the discrepancy between the velocity of the foot, peak and tail of that wave. If the wave is very slowly developed, then, all parts should be propagated at nearly the same velocity. The difficulty in reading the actual first pressure rise in such a slowly developing pressure wave

TABLE 1

Velocities of a pressure wave and the period of the standing wave in a Gooch tube of 21 cm. length, as related to the rate of pressure development

(Diastolic pressure constant at 50 mm. Hg)

NUMBER OF PULSES	AVE. RATE OF PRESSURE DEVELOPMENT	AVE. TRANSMISSION TIME, WAVE FOOT	AVE. STANDING WAVE HALF-PERIOD	PREDICTED* TRANS. TIME, WAVE FOOT
	mm. Hg/m.sec	*m.sec.*	*m.sec.*	*m.sec.*
9	0.5	30.0	37.3	30.0
9	0.8	28.2	35.6	29.0
9	1.2	28.5	36.4	30.2
9	1.6	28.4	39.2	27.6
9	2.5	24.6	38.4	24.7
9	3.4	22.0	37.2	23.8
9	5.0	18.0	38.0	19.1

* The predicted transmission time, using formula III, from the slowly developed extensibility curve of the wall of the tube, was 35.6 m.sec., which agrees well with the half-standing wave period. The predicted transmission times given above were derived by formula III from the respective extensibility curves at the corresponding rates of tension increase.

prevents the exact proof of this statement, but the data clearly indicates its likelihood. With such a slowly developed wave, a symmetrical standing wave would be developed only if the wave generated happened to have a length twice that of the tube. With faster rising pulses, the differences in velocities of the various parts cause a lengthening of the wave until it reaches a value of twice that of the tube. The number of trips through the tube that are required before this symmetrical standing wave is established will depend upon the length of the pressure wave generated, and the rate at which pressure was developed.

Because the stretch and recoil curves, upon repeated stretches, come to the straight line relationship obtained with a slowly developed stretch, the velocity of the peak of the propagated wave, once it has reached a constant value, should be predictable from this slow stretch line by the use of equation III. Experimental data show this to be true. So long as the hysteresis loop is present, the

velocity of the foot of the wave cannot be predicted from the slow stretch line. Nor is the initial velocity of the foot an index to the period of the standing wave which later will be developed.

III. *The pulse wave velocity in the living dog.* The transmission times of normal pulse waves (timing from the point of first pressure rise) have been ascertained, from the ascending aorta to the fork of the iliacs, in animals whose diastolic pressure values have been varied from 20 to 170 mm. Hg through injections of epinephrine or acetylcholine. The correlation between these transmission times and diastolic pressure for a representative animal is shown in figure 6. While the relationship shows a slight curve, the deviation from a straight line is nowhere pronounced. If these transmission times are converted to pulse wave velocities, the derived curve is qualitatively similar to those published by Bramwell and Hill (3) and by Steele (13), differing however in the amount of curvature exhibited in different pressure ranges. Such variations are not surprising in view of the variety of arteries represented in the three studies. Our measurements indicate that the relationships are generally not the same for all parts of the aorta. The thoracic aorta does show a fairly constant pulse wave velocity at low diastolic pressure levels, while the abdominal aorta does not. When the wave has progressed through the whole length of the aorta, however, this effect of the thoracic aorta has been largely lost.

IV. *The velocity and contour changes of artificially generated pulse waves in the dog aorta.* Immediately upon sacrifice of the dog, the aorta was exposed by evisceration, and all arterial branches tied off but wherever possible left intact. Hence the aorta was left *in situ* as a blind sac, terminating at the fork of the iliacs, with a very slow drainage. Injection pulses were made into this sac through a large glass cannula tied into the root of the ascending aorta. The tubing connections in the line of flow from the pressure burette were carefully selected not to present constriction which might cause turbulence.

Artificial waves generated in this system showed the same quantitative relation (crosses, fig. 6) between the velocity of the foot of the wave and the diastolic pressure level, as did normal heart pulses. Hence the characteristics of the system had not been significantly changed in this respect by the death of the animal. Further, it indicated that the stream velocity did not accelerate the natural pulse wave velocity sufficiently to make it greater than the random error of observation between various waves.

A striking similarity between the transformation of a pressure wave with repeated trips through the Gooch tube and through the aorta is apparent (fig. 7). Both systems show a difference between the transmission time of the foot and the peak of a propagated wave. Both show a progressive slowing of the peak velocity to a constant figure, which value also establishes the period of the standing wave. In both, the attainment of a symmetrical standing wave occurred after the transformation of the propagated wave into one whose length was twice that of the tube. In both, this symmetrical wave was developed only after the foot of the oncoming wave had over-run the lagging tail of the preceding wave. In both, the velocity of foot and peak proved independent of the size

of the pressure wave generated. The differences between the two systems were: 1, the foot velocity of the aorta, on its first trip, proved largely independent of the rate of pressure development (table 2); 2, a constant velocity of the peak of the wave was attained more rapidly in the aorta, and a symmetrical standing wave was often developed after three or four trips of the propagated wave; 3, the

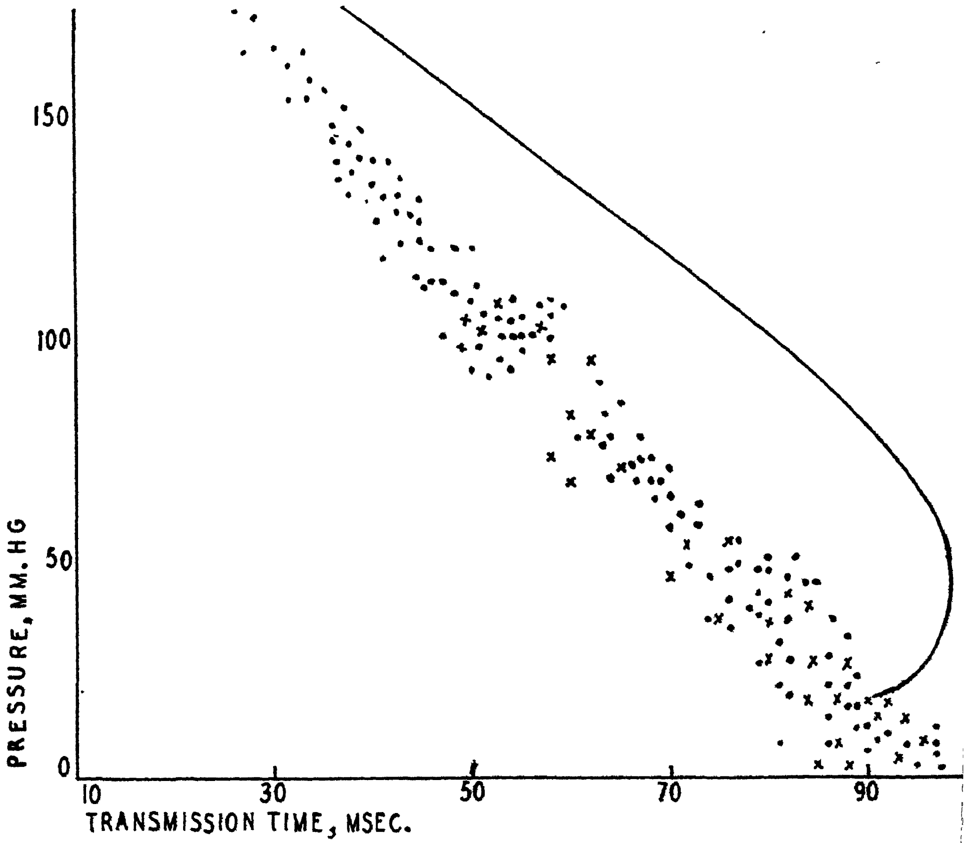

Fig. 6. The relation between the transmission time of the pulse wave, between ascending aorta and the bifurcation of the iliacs, and the diastolic pressure. The plotted points represent actual values obtained in a living animal when diastolic pressure level was varied by means of injected epinephrin or acetylcholine. The crosses show the transmission time for artificial pulses, made from a pressure burette, into the tied off aorta, post mortem, left *in situ*. The plotted curve represents transmission times calculated from the tension-length relations for the rings of the aorta, as developed by a slow stretch, using formula III.

velocity of the tail of the wave was relatively slower than with the Gooch tube; so much so in fact, that the identification of a distinct end of the wave was often impossible. This greatly retarded tail was most noticeable in the ascending aorta, least in the abdominal aorta. Hence the top of the aorta was never free of a pressure wave from the time of the first pressure rise, so that the passage back and forth of the propagated wave could be followed only with difficulty. These

facts serve to emphasize the fact that the aorta is not a homogeneous elastic system, and that the step-wise transformation in form of the parts of the pulse wave cannot be easily predicted.

V. *The measurement of the elastic properties of the aorta.* The aorta was measured *in situ* from the aortic valves to the fork of the iliacs, determining the distance between such landmarks as the renal, segmental and mesenteric arteries. After removal from the body as a single unit, the aorta was remeasured. It was assumed that the longitudinal relaxation did not affect the stretch characteristics of the circular fibers. Actual observations indicate that while this assumption is not strictly valid, the error involved is not large. For the calculation of volume-pressure relations, therefore, the *in situ* rather than the relaxed length is used.

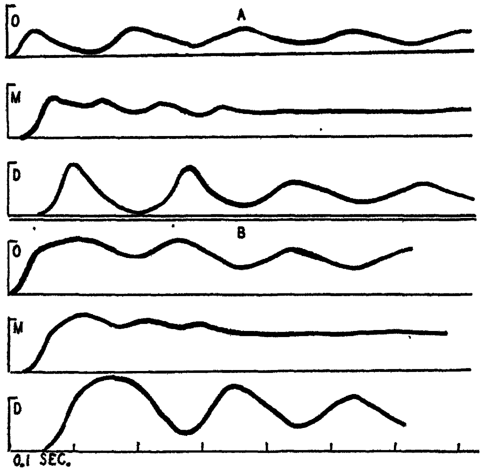

Fig. 7. The transformation of an artificial pressure wave in transit through a dog aorta. O, record taken from the ascending aorta, near injector cannula; M, record from the lower thoracic aorta; D, record from the abdominal aorta just above the iliacs.

The excised aorta was then cut serially into rings of 10 mm. length, each ring being subjected to tension separately. Half-circumference values were converted into internal volumes, and the tension calculated to units of internal pressure. The pressure-volume relations so obtained were plotted and the slopes of this curve at different pressure levels determined by means of a tangent meter. From these tangents, by means of formula III, the expected wave velocities were derived. The volume of, and the transmission time for, the whole aorta were then obtained by the addition of the values for the separate segments.

Reference to figure 6 shows that both a qualitative and quantitative discrepancy exists between the predicted curve for the pulse wave velocity and that showing the velocity of natural and artificial pulses. At diastolic pressure levels over 70 mm. Hg, the difference appears quantitative only, and can be corrected by the substitution of a constant of 0.6–0.7 for the Moens factor of 0.9. Below a diastolic pressure of 70 mm. Hg, the difference seems to be qualitative as well.

Likewise, within diastolic pressure levels of 70 and 150 mm. Hg, the final con-

stant velocity of the peak of an artificially generated pulse wave in the aorta could be reasonably predicted from the slow stretch curve for the wall for formula III, but below 60–70 mm. Hg, such predictions were in error.

As with the Gooch tube, the stretch characteristics of the aortic rings at different rates of tension development were obtained. Several points of interest were revealed. First, the tension-length curve of the aorta was modified but little by changes in the rate of application of tension within the limits corresponding to 0.2 and 10 mm. Hg per m.sec. While these curves did not differ significantly from each other, they were uniformly of a different form from that shown when

TABLE 2

The independence of the velocity of an artificial pulse and the rate of pressure rise in an aorta, with branches tied off, but left in situ

(Diastolic pressure constant at 65 mm. Hg)

NUMBER OF PULSES	AVE. RATE OF PRESSURE DEVELOPMENT	AVE. TRANSMISSION TIME	AVE. STANDING WAVE HALF-PERIOD
	mm. Hg/m.sec.	*m.sec.*	*m.sec.*
7	0.6	42.1	58.8
8	1.2	41.5	60.0
8	1.8	40.4	58.2
7	2.8	42.8	61.2
6	5.5	41.5	60.1
5	8.7	40.3	57.0
Mean		41.4	59.2

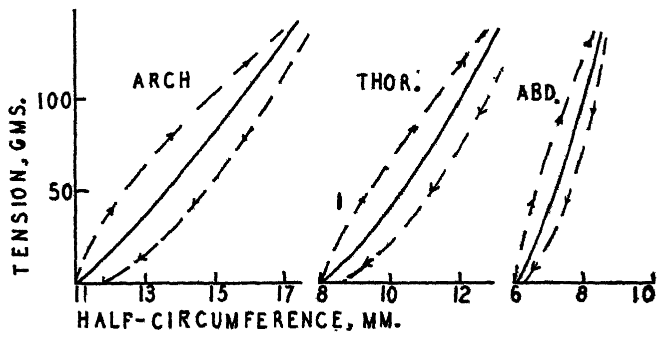

Fig. 8. The effect of rapid stretching upon the extensibility curves of rings taken from the arch, thoracic and abdominal portions of the dog aorta.

the aorta was stretched slowly by the Scott serigraph (fig. 8). As with the rubber, a conspicuous bowing was present at the beginning of the stretch curve, and, as with the rubber, pulse wave velocities calculated on the basis of the first part of this rapid stretch curve by means of formula III adequately explain the accelerated velocity of the foot of a propagated wave. How slow the rate of tension development would have to be to have the slow and rapid stretch curves merge was undetermined—at least the necessary rate is too slow to be of physiological importance.

As with the rubber, repeated rapid stretches, even though the rate of tension

development remained unchanged, modified the stretch curve so that it soon became identical with that shown when the aorta was stretched slowly. The recoil curve, however, remained qualitatively different throughout many stretches. More so than with the rubber, the aortic wall showed a reluctance to assume its original diameter. Velocities predicted from this sluggish return would indicate a very slow "tail" of the wave, a finding already noted for the artificially generated waves.

Since the distensibility curve is not modified greatly by the rate of tension development, the Bramwell-Hill formula can be modified so that it will predict the velocity of the foot of the wave by use of a constant of the order of 0.6 to 0.7. As pointed out previously, however, such a correction would be valid only when the diastolic pressure was over 70 mm. Hg.

Discussion. A review of the experiments previously published in which agreement between the velocity of the foot of a propagated wave and the elastic properties of the wall was established, reveals that in most cases mercury was used as the fluid medium. Since the transmission time is appreciably longer when mercury is used, it can be measured more readily. While it should be possible to produce a pressure wave at the same rate of speed with mercury as with water as the medium, the practical difficulty would be considerable. A slower rate of tension development than those used here, which were chosen to be comparable to the pressure rise in the living animal, would render smaller the discrepancy between observed foot velocity and that predicted by the Moens formula, and also the discrepancy between foot velocity and the period of the standing wave.

It is realized, too, that the aorta is not a homogeneous system throughout its length. More consistent results might have been obtained with but a short segment of the aorta, e.g. the thoracic aorta. Since even the whole length of the aorta is but a part of the Windkessel, any modification for purpose of stroke volume determination should be toward the inclusion of arterial branches, with a more heterogeneous system, rather than a restriction to a single part.

Little, however, has obtained agreement between the velocity of the foot of a pressure pulse and the predicted velocity in a homogeneous rubber tube filled with mercury. The fact that this tube was extremely long presents another problem. As a wave is propagated through a tube, a part of the total energy is being continually lost as heat, i.e. damping. This energy loss will be reflected by a progressive lowering of the peak pressure value, such as has been consistently seen in the records reported. Decrease in the pressure height without a change in the wave length would be equivalent to a progressive decrease in the rate of pressure development, which would, in turn, make the distensibility curve more and more similar to that found with the slow Scott serigraph. If the tube were long enough (Little used 50 ft.), the foot and peak would presumably be moving at the same speed as they neared the end of the tube, and at a velocity which could be predicted from a slowly developed tension-length line.

SUMMARY

1. When a short wave is generated in either the aorta or in a length of Gooch tubing, the start or foot of the wave is transmitted at a high velocity, the peak

of the wave at a slower velocity, and the "tail" of the wave at a velocity slower still. These relationships continue as the wave is reflected back and forth between the ends of the tube.

2. None of these velocities can be calculated by the classical formulae from the tension-length relations which are shown when circular rings of the tube are stretched slowly. If the stretch is made rapidly, there is enough viscous resistance to elongation to account quantitatively for the rapid propagation of the initial parts of the pulse wave, and enough viscous interference with recoil to account quantitatively for the retarded velocity of its later parts.

3. The fact that the initial foot of the pulse wave travels faster than the peak and the later parts of the wave, means that there will be a progressive lengthening of the wave as it is propagated through the tube, the initial parts begin to overlap the later parts, and the oscillations at the ends of the tube become more and more like those of a standing wave. During this time the peak of the wave is being propagated more slowly, reaching a final constant velocity which establishes the period of the standing wave. The period of the standing wave can be calculated from the tension-length relationship exhibited when the tube is slowly stretched. This is true because a shift in the tension-length curve follows repeated stretching at short intervals, so that the viscosity shown on the first stretch is decreased, until it is no greater than that seen with a slow stretch.

4. The velocities of the various parts of the propagated wave are dependent solely upon the basal volume level, and are independent of the size of the pressure pulse generated. The velocities of the three parts of the wave depend, then, upon the three tangents to three separate volume-pressure curves at their departure from the initial volume level. The tangent of the distensibility curve with rapid stretch establishes the speed of the foot of the wave. That of the recoil curve following a rapid stretch, establishes the speed of the tail of the wave. The tangent of the volume-pressure curve obtaining when tension is slowly applied determines the final, constant velocity of the peak of the wave, and hence the period of the symmetrical standing wave.

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SOME DIFFICULTIES INVOLVED IN THE PREDICTION OF THE STROKE VOLUME FROM THE PULSE WAVE VELOCITY[1]

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The distensibility of the elastic arterial tree, if it could be described in quantitative measure, would be a good index to the stroke volume of the heart (1–3). For the stroke volume (SV) must be

$$SV = Vs - Vd + Ds$$

where Vs and Vd are the volumes of the arterial reservoir at the end of systole and of diastole, and Ds is the drainage during systole. This last can be rather safely predicted, for drainage is proportional to the pressure existing above 20 mm. Hg (4), and the drainage during diastole should equal the aortic uptake. By the respective areas under the systolic and diastolic portions of the pulse curve, therefore, the drainage during systole can be calculated from this diastolic drainage level.

The immediate problem, then, is the gauging of the volume uptake of the aorta as the pressure rises during systole. Several methods for this estimation have been advanced, based on the velocity of the arterial pulse wave, which is also conditioned by the distensibility of the wall. The basic formula usually applied is the Bramwell and Hill (5) "transformation" of the classic equation of Korteweg (6), in which

$$Vp = 0.357\sqrt{V\Delta p/\Delta v}$$

where Vp is the pulse wave velocity in meters per second, Δp is the rise in pressure in millimeters Hg corresponding to Δv, the increment in volume in cubic centimeters, starting from V, the diastolic volume of the tube.

Such an equation predicts that the pulse wave velocity will be a constant index to the filling curve of the aorta. We have shown, however, that because the walls of the aorta have an internal viscosity, the distensibility curve is distorted at its very start when tension is applied rapidly. The speed of the foot of the pulse wave is accelerated by this decreased distensibility at the start of the curve; the total filling curve, however, is not seriously affected. It is necessary, therefore, to introduce an empirical factor into the Bramwell and Hill formula which will relate the two distensibility curves to each other. In other words, the pulse wave velocity can never be a quantitative measure of the total volume uptake in the living animal. If the degree of distortion is small, and varies but little with the rate of tension development, i.e., if the correction factor is almost constant, then, for practical purposes, a formula

[1] The expenses of this investigation were partly defrayed by a grant from the American Medical Association.

might be developed which would allow use of the wave velocity as an index to the filling curve. In general terms, such an equation would be $Vp = 0.357 F \sqrt{\Delta p V / \Delta v}$, where F would be the empirical factor to cover the influence of wall viscosity.

When an evaluation of the F was attempted (7), it soon became clear that it depended in part upon the diastolic pressure level. Above a level of 70 mm. Hg, a value of 0.6–0.7 for the dog, and 0.8 for the human, would allow a fairly safe estimate of the relative distensibility of the aorta from the pulse wave velocity. Below this pressure range, the factor becomes much more variable. It is as though the physiological construction of the aortic wall had changed in going from one pressure range to the other.

Now it is well recognized that the aortic wall has three systems, all of which are distensible. The tension-length relation of smooth muscle, divorced from connective tissue, is of course not known. Krafka (8) found the tension-length

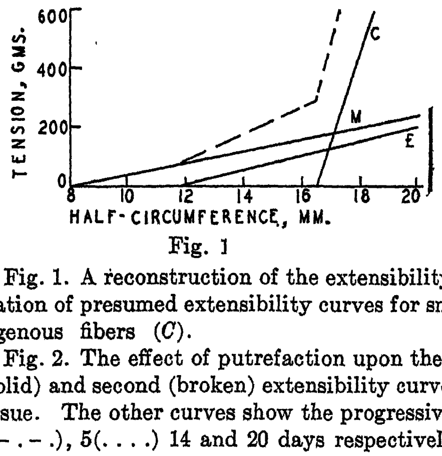


Fig. 1

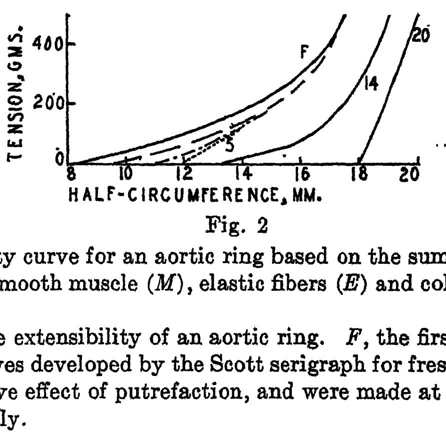


Fig. 2

Fig. 1. A reconstruction of the extensibility curve for an aortic ring based on the summation of presumed extensibility curves for smooth muscle (M), elastic fibers (E) and collagenous fibers (C).

Fig. 2. The effect of putrefaction upon the extensibility of an aortic ring. F, the first (solid) and second (broken) extensibility curves developed by the Scott serigraph for fresh tissue. The other curves show the progressive effect of putrefaction, and were made at 2 (. – . – .), 5(. . . .) 14 and 20 days respectively.

relation for ligamentum nuchae, and for tendon to be linear. If we may assume that linear relations hold for each of the three systems, then the complex extensibility curve of an aortic ring could be derived on the basis of three straight lines, starting by necessity, from different initial length values (fig. 1). To avoid sharp inflection points, one need assume only that the transition from one tissue type to another, as the wall is stretched, is not abrupt.

Such an analysis indicates that there should be a pressure range, starting from 0, where the distensibility of the wall would be reflecting the elasticity of but a single one of these three possible systems. It seems obvious that the least distensible of the three systems would be the collagenous fibers, which means that the marked steepening of the aortic distensibility curve at high pressure levels would mark the high resistance to stretch of the outlying collagenous coat. There are several indications that the first of the systems involved is the smooth muscle.

1. From the time of MacWilliam (9) it has been recognized that the exten-

sibility of the aortic wall may be altered by various mechanical and chemical stimuli. Upon exposure to air and removal from the body, the rings appear thicker, firmer and smaller. Upon manipulation, this appearance of contraction is lost, and, according to the earlier measurements, the wall becomes increasingly extensible. Epinephrin renders the wall more resistant to stretch, and a variety of agents known to cause relaxation of smooth muscle, will render it more extensible (9). A similar conclusion is evident from the results of Krafka (8), who found that a first stretch would suffice to cause relaxation of the muscle elements, and leave the wall more extensible for a second stretch.

When various aortic rings from our dogs were subjected to stretch by a Scott serigraph (8), it was found that this difference in extensibility between the contracted and relaxed aorta was largely artificial. For upon completion of the first stretch, and during the half-minute rest period allowed between stretches, the ring did not return to its initial half-circumference value. If the initial pin separation was not reset to the new level, slippage followed the first application of tension, which appeared on the record as a relatively great extensibility, such as indicated by the records of MacWilliam and Krafka. If the initial pin separation was reset to bring the once stretched ring tight, the second stretch curve ran almost parallel to the first through the first tension increments, reached an inflection point at about the same absolute half-circumference level, and then gradually approached the first curve. By the time full tension was developed, the two curves were identical. Several points seem indicated. First, that muscle was the system involved in the first part of the extensibility curve; second, that the extensibility of contracted and relaxed muscle were not greatly different; and third, that the greatest change as the result of muscle contraction was an absolute decrease in the initial length, with the corollary that the pressure level at which the inflection in tension-length curve will be effective will be higher.

If, upon completion of the second stretching, the ring was placed in 1/10,000 epinephrin solution, the initial half-circumference value became smaller, although it did not reach the level existing before the first stretch. A much smaller contraction would develop if the ring were merely allowed to remain in Ringer solution for several hours. Acetylcholine did not apparently relax further a once stretched ring, but it did prevent the contraction seen in Ringer solution. This might mean simply that upon completion of a stretch, the smooth muscle was completely relaxed.

2. MacWilliam (9) further showed that as the aorta was allowed to putrefy, the extensibility curve was progressively changed. On repeating this experiment, we allowed several segments of aorta to putrefy in Ringer solution for a period of 22 days. Every 48 hours, two rings were sliced off the end of each segment, one of which was subjected to a series of stretchings, and the other fixed and sectioned for histological study.[2] From a survey of the respective stretch curves (fig. 2), it would seem that the initial strong contraction which

[2] The histological data included is from a report by Dr. E. R. Pund of the Department of Pathology, who sectioned, stained, and studied the various putrefied rings.

followed removal of the vessel from the body was gradually lost over the first 72 hours. After this time, first and second stretches largely gave identical curves. During this same time interval, the starting half-circumference was gradually increasing, until it was finally larger than with the second stretch on on fresh tissue. Histological analysis showed that in the same interval, the muscle cells were losing their nuclei and gradually disappearing, but that both collagenous fibers and elastic tissue were unchanged.

As putrefaction advanced beyond the fifth day, the stretch curves now became quite constant, showing a slope parallel to the middle region of the curves obtained on fresh tissue, i.e., the "musculo-elastic" portion. Around the 15th day, the extensibility curve changed again, being more extensible in the lower increment regions, but steepening again at the higher tension levels. Sectioned tissues showed that at this time the elastic fibers of the intima and adventitia had disappeared, but those of the media were still relatively intact, although losing in part the continuity of a sheet of tissue. Collagenous fibers were still present in large numbers, although some were swollen and homogeneous.

The experiment was terminated at 22 days, for by this time the ring would rupture before a definite stretch curve could be determined. Such rings showed an almost complete disappearance of elastic tissue, and a thinning of the collagenous layer with the assumption of an amorphous and granular appearance.

From these two lines of data, therefore, it seems justified to say that the first part of the extensibility curve is conditioned almost exclusively by muscle, the middle phase, by muscle and elastic tissue, and the higher tension range, by all three systems. In terms of absolute pressure values, the inflection points in the stretch curve vary according to the location along the aorta. The shift from a muscle to a musculo-elastic system is at around 70 mm. Hg. in the ascending aorta and arch, 60 mm. Hg in the thoracic, and 40 mm. Hg in the abdominal aorta. These levels are, of course, not fixed, but will be varied by any change in contraction of the muscle. They are based upon the second stretch curves performed on fresh tissues. The stretch characteristics of the different aortic rings allow a reasonable grouping into three general regions, with but minor deviations within a group. In figure 3 are shown the summated pressure-volume relations for the three groups, i.e., the ascending aorta and arch, the thoracic, and the abdominal aorta. The differences between these three curves are strikingly similar to those noted (10) when the aortas of subjects from different age groups are compared. The ascending aorta is comparable to the "young" aorta and the abdominal to the "old". Further, as the ascending aorta putrefies, its distensibility curve becomes more and more like that of the "old" aorta, with its larger starting volume and its decreased distensibility.

Now if a change in muscle contraction can so seriously affect the extensibility curve and hence the volume-pressure relations of the aorta, any estimate of absolute aortic distensibility in the living animal must be based on a quantitatively accurate measure of the existing diastolic size. In view of the marked contraction of our rings upon exposure to air, autopsy measurements would seemingly be valid only if the aorta normally shows a comparable contraction

in life. We have satisfied ourselves that the aorta left *in situ* in the dead dog is relatively relaxed. When the same aorta is cut into rings, it is relatively contracted.

The volume of the aorta *in situ* was even greater than that calculated from stretch curves. The vessel was exposed by evisceration, all branches tied off but wherever possible left intact, and a large cannula tied into the ascending aorta at the region of the aortic valves. Known volumes of salt solution were then injected into this blind sac from a pressure burette. Pressure changes corresponding to the known volume injections were recorded by means of sounds, placed in the abdominal aorta and arch, connected to optical manometers.

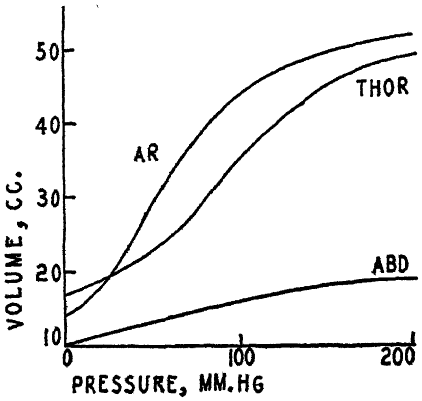


Fig. 3

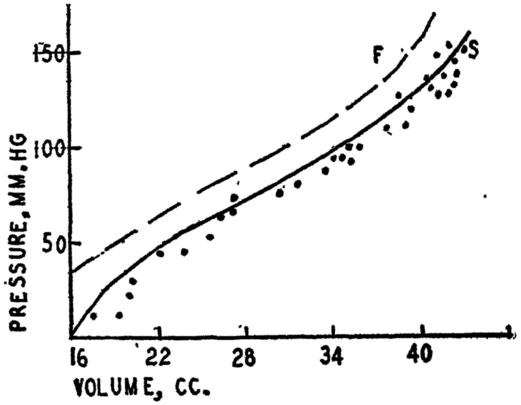


Fig. 4

Fig. 3. Volume-pressure relations for the ascending aorta and arch (*AR*), the thoracic, and the abdominal aorta for the dog, as calculated from extensibility curves of the various rings subjected to slow stretch.

Fig. 4. Volume-pressure relations for the *post mortem* aorta of the dog, left *in situ*. Volume-pressure increments for artificial pulses are plotted as points. The solid line represents volume-pressure relations for the whole aorta as calculated from second stretch curves of the various rings. The broken line, the relations as calculated from the first stretch curves.

Drainage from the system, which was small, was estimated from the rate of pressure fall in "diastole".

At the conclusion of the experiment, the aorta was removed, cut serially into rings, and each ring subjected to stretch. It was assumed that the volume derived from the stretch curves corresponding to a pressure of 180 mm. Hg was correct (the first and second stretch curves were then, in greatest part, identical, and conditioned largely by collagenous fibers) and the actual volume-pressure increments referred to this derived 180 mm. Hg pressure volume level. As shown in figure 4, the conclusion must be drawn that the aorta in the dead dog, when subjected to repeated stretchings by administered artificial pulses, was even more relaxed than were the individual rings upon a second stretch.

Whether the aorta in the living animal is likewise relaxed is most uncertain.

Probably the best direct evidence on the question of how extensive tone changes may be in the aorta is that of Wiggers and Wegria (11) who, using an aortograph, showed a decreased aortic size, not accompanied by a change in arterial pressure, following the injection of epinephrin. Optically recorded pulse contours in the turtle show a decrease in capacity and change in the elastic properties of the pulmonary reservoir after an injection of epinephrin, with no change in the pulmonary peripheral resistance (12). Evidence in support of a specific increase in volume following the use of vasodilator drugs or vagal stimulation, is not conclusive (13). The problem admittedly is a difficult one to solve.

It is customary, in evaluating the diastolic volume of the human aorta, to refer to the aortic sizes given by Bazett and associates (1) as related to age and surface area. For example, Cournand *et al.* (14) referred their index of diastolic size, as determined from x-ray pictures of the ascending aorta in subjects given diodrast, to the Bazett figures, concluding that the actual volumes in life were much larger. It should be recalled, however, that Bazett states that the figures "as expected give too low an estimate of the area of the aorta distended at its working pressure". Any use of these figures to give an index to diastolic size is, of course, totally unwarranted. We have shown elsewhere (15) that a human aorta increased its area some three-fold when going from an internal pressure value of 0 to 80 mm. Hg, an increase even smaller than that found by Hallock and Benson (10). Cournand's figures, therefore, would indicate not a larger vessel, but a strongly contracted one.

In their study on the estimation of the stroke volume from the pulse wave velocity, Bazett *et al.* (1) give formulae from which can be derived the diastolic size not only of the aorta but of the arterial branches as well. It should be borne in mind, however, that these formulae were derived indirectly. The aortic volume is based upon the zero pressure aortic cross area, which is to be increased by a given correction factor, which includes in itself an estimate of aortic length. This factor was merely chosen so that when the volume so obtained was multiplied by a distensibility index, with a drainage component added, the total stroke volume derived would be equal to that determined by the acetylene method. The distensibility index was derived from recorded pulse wave velocities through the arteries. As we have shown elsewhere (16), the pulse wave velocities, at least for the ascending aorta, are probably too small. The stroke volume given by the acetylene method is also probably too small (17). Hence the Bazett formulae, derived solely to allow a fit with a given set of data, are hardly inviolable as indices to diastolic size.

When the diastolic volume of a human aorta which we have studied (15) was assembled from the stretch records of the various rings, the value obtained was 1.5 times greater than the Bazett formulae would predict for the aorta and the arterial branches as well. It should be emphasized that we do not claim that our figure is any more pertinent to the problem than are the estimates of Cournand and of Bazett. It seems clear, however, that we are left in the perplexing state in which no single estimate of the actual diastolic volume in life can be regarded as accurate. Since tone changes are entirely possible in the living

animal, and since diastolic volume can thus be varied, any quantitative measure of the aortic uptake from any known index is most insecure.

The problem of how changes in muscle tone would affect the pulse wave velocity is very complex. Analyzing our previous statements as to the variability of the empirical viscosity factor F, in the light of our interpretation of the aortic stretch curve, it would seem that the nearer the stretched length approached the point at which the outlying collagenous fibers largely condition the distensibility, the less variable the *F* value. Conversely, the greater the rôle of muscle in the determination of the wall distensibility, the more erratic is the *F* value. This effect was studied more carefully by means of a rapid stretch apparatus (7) with which stretch curves were developed from initial levels well within the range at which muscle was the most important distensible component. It was apparent that the lower the initial setting, the greater the discrepancy between the rapid stretch curve (pulse wave velocity) and the slow stretch curve (aortic filling). In absolute terms, however, the initial slope of the rapid stretch curves remained almost constant until the inflection point between muscle and the musculo-elastic systems had been reached, when it was increased. Now a constant slope in the face of an increasing diastolic volume would serve to give an ever increasing pulse wave velocity as the diastolic pressure rose. We have already shown (7) that such a relation between pressure level and pulse wave velocity holds for the living animal. The application of the Bramwell and Hill formula to the filling curve in this low pressure area would indicate just the opposite, a decreasing or a constant velocity as the diastolic pressure rose (fig. 7).

While a qualitative accord between velocity values actually measured and as derived from rapid stretch volume-pressure relations, is easily obtainable, a strict quantitative relation between the two is more uncertain. It was found that the initial half-circumference value at a given pressure setting could change through such wide limits that calculated velocity figures varied markedly. If a ring was allowed to remain under tension for several minutes, it gradually enlarged to a value greater than that indicated by the second slow stretch curve. Once it had seemingly reached a constant value, it would usually not return to the same initial level when subjected to a series of stretches. The problem is reduced, then, to the selection of a series of diastolic sizes which are comparable, and from which pulse wave velocities can be derived. When, for example, the diastolic size was taken to be the largest reached by a ring after prolonged stretching, the calculated pulse wave velocity was not greatly different from the actual value. What does seem explicable is the fact that the scatter of individual points in a measured pulse wave velocity against diastolic pressure plot becomes much greater at the low pressure levels.

Because the walls have an internal viscosity, the aortic ring not only shows a decreased extensibility at the start of a stretch, but also a sluggish resumption of its initial size after tension is withdrawn. In figure 5, for example, is shown an extensibility curve developed by a rapid stretch, in which both the increase in tension and half-circumference are plotted against time. In this case, the

initial half-circumference value would not have been reached until some 500 m.sec. after all tension was removed. When another stretch was begun before the completion of this period of return, the second extensibility curve started from a higher level. This would indicate that both the aortic distensibility and the pulse wave velocity could be dependent in part upon the time interval

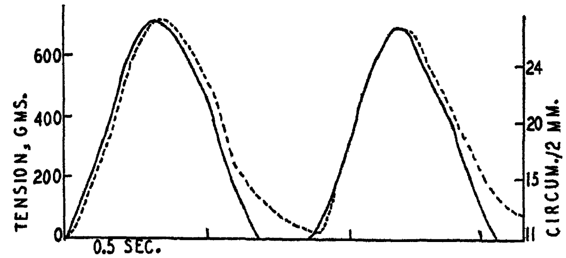
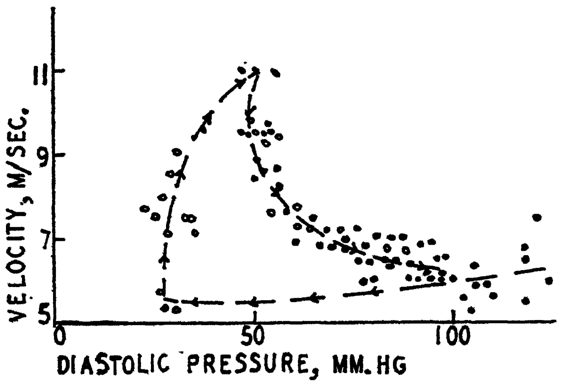


Fig. 5. The extensibility and recovery curves for an aortic ring subjected to rapid stretching. The solid line represents the change in applied tension, plotted against time. The broken line, the change in half-circumference value—the ordinate denoting half-circumference has been so constructed that the tension line will also denote the half-circumference values which would obtain if the ring were stretched slowly (Scott serigraph).



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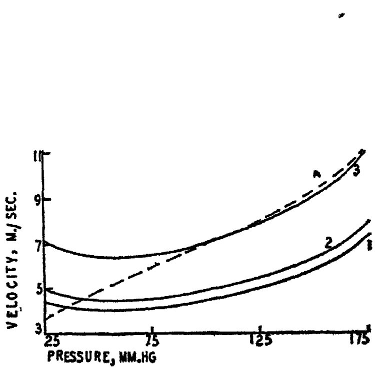

Fig. 7

Fig. 6. The relation between pulse wave velocity and the heart rate of the dog. The solid circles represent velocity values obtained in the control period. An injection of acetylcholine was then made, the pressure and velocity values both changing. The line shows the sequence of readings, as the heart rate first slowed, and then accelerated.

Fig. 7. The relation between pulse wave velocity and diastolic pressure in the dog. The broken line denotes the change in velocity with pressure rise in the living animal. The solid lines show values which are calculated from the slow stretch curves for aortic rings, using formulas 1, 2 and 3.

allowed between stretchings, i.e., upon the heart rate. That such a conclusion is physiologically sound might be deduced from the following experiment. Recording sounds were placed in the arch, and also passed up through the femoral artery to the region of the diaphragm, in a living dog. The pulse wave velocity through the descending arch and thoracic aorta could thereby be measured.

In the control period, the pulse wave velocity was fairly constant at around 6M. per sec., and the pulse rate was such that from the first pressure rise of one pulse to the first rise of the next was 800 m.sec. Acetylcholine was then injected, resulting in a fall in diastolic pressure and an initial slowing of the heart rate. The first slow beats recorded (fig. 6) showed a velocity of 5.5M. per sec. The interval between these heart beats was 8.5 and 15 seconds respectively. Then, as the pulse rate increased, the pulse wave velocity also increased, even though the diastolic pressure remained unchanged. At one point the velocity reached 11M. per sec., at which time the interval between pulse beats had been decreased to 450 m.sec. The explanation seems clear. With the fast heart rate, not enough time was allowed between successive heart beats to allow a complete return of the aorta to its original diastolic volume. A new stretch started, then, from a higher level, and as a result, the pulse wave velocity was increased. The reciprocal effect, i.e., a slowed pulse wave velocity with decreased heart rate, following vagal stimulation, has been previously reported (18). We might expect that this dependence upon the pulse rate would be greatest at the lower pressure levels, where the distensibility of the aorta is greatest, and where the greatest discrepancy exists between the rapid and slow extensibility curves.

A brief review of the formulas which have been developed in this and a previous paper (7) will serve to summarize the above data. In an attempt to relate the pulse wave velocity to the volume-pressure relations (filling curve) of the aorta, Bramwell and Hill (5) reexpressed the theoretical equation of Korteweg (6) to read

$$Vp = 0.357\sqrt{\Delta pV/\Delta v} \tag{1}$$

Working at the same time as Korteweg, Moens (19) developed, as a result of actual experiments, the same formula, except that it bore a correction factor of 0.9 which he treated as a constant. We have shown (7) that, when such a factor is used, the formula will predict with reasonable accuracy the period of the standing wave in the arterial system. Hence

$$2L/S.W. = 0.357 \times 0.9\sqrt{\Delta pV/\Delta v} = 0.32\sqrt{\Delta pV/\Delta v} \tag{2}$$

where $S.W.$ is the period of the standing wave and L the length of the aorta. The use of such a formula to estimate the stroke volume is limited for two reasons: 1, because of possible muscle contraction, the diastolic volume of the aorta cannot be merely assumed from autopsy data, or from stretch curves. Nor can this volume be derived by any known techniques; 2, the period of the standing wave is very difficult to determine accurately from pulse contours.

A relation of the pulse wave velocity to arterial filling is even less sound. The application of equation 1 to the filling curve gives velocities much lower than actual (fig. 7), and, at low pressure levels, would indicate a reversal of the trend for an increasing velocity with an increasing diastolic pressure. The equation must include an empirical factor which will correct for the internal viscosity of the walls, which in turn, depends directly upon the structural ele-

ment in the wall which is governing its extensibility at the moment. In the higher ranges of diastolic pressure, an approximation can be had by:

$$Vp = 0.22\sqrt{\Delta pV/\Delta v} \quad \text{for the dog} \tag{3}$$

and

$$Vp = 0.27\sqrt{\Delta pV/\Delta v} \quad \text{for the human.}$$

In the lower pressure ranges, no constant correction factor will suffice. The difficulty is twofold; 1, the discrepancy between rapid and slow stretch curves becomes greater as the pressure is lowered, and 2, the diastolic volume fluctuates widely, as the degree of muscle contraction is changed, and also as the rest period between successive heart beats, i.e., the pulse rate, is changed. Hence we must conclude that the estimation of a stroke volume from the pulse wave velocity is on insecure grounds.

SUMMARY

The calculation from pulse wave velocity of the distensibility of the arterial tree, as a step in estimating the stroke volume of the heart, requires the determination of the effect of the viscous elastic properties of the arterial wall and an estimation of the diastolic size of the arterial reservoir.

The aortic wall is a heterogeneous elastic system, with different viscous properties for each of the contained elements. Contraction of smooth muscle may alter the relative rôle of each of these elements in determining the resistance of the wall to stretch. The diastolic size of the aorta will vary not only with the tone of the smooth muscle but also with the time allowed between heart beats for the sluggish return of the wall to its original diastolic size.

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THE CONSTRUCTION OF A THEORETICAL CARDIAC EJECTION CURVE FROM THE CONTOUR OF THE AORTIC PRESSURE PULSE[1]

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In the course of a series of recent investigations which showed that the velocity of the arterial pulse wave cannot be used as a measure of the volume uptake of the aorta in systole (1, 2), extensive data were gathered on the pulse wave velocity and the volume-pressure relationships of the aortas of some 14 dogs. From such data we have attempted to construct cardiac ejection curves which would produce a given conformation of the central pulse. It should be recognized that such a reconstruction cannot be used as a quantitative measure of stroke volume, particularly in the lower pressure ranges, for the diastolic volume which obtains in the aorta cannot be directly predicted from autopsy data. Despite its quantitative limitations, the reconstruction of a cardiac ejection curve does lend insight into several fundamental phenomena involved in cardiodynamics.

The approach differs in detail from that used by Otto Frank in developing the "Grundform des arteriellen Pulses" (3) in that the time relations of the distention of successive segments of the aorta by the pulse wave were worked in quantitatively. The aorta is thus not considered as a single compression chamber or Windkessel but rather as a tube whose parts are successively distended.

Volume-pressure relations of the various aortas with which we have worked were assembled by calculation from the tension length curves obtained when aortic rings, cut serially, were stretched by a Scott serigraph (4). Body weights of the animals used varied from 7 to 17 kgm., averaging 14.5 kgm. The average length of the aorta *in situ*, from aortic valves to bifurcation of the iliacs, was 326 mm. It has been shown (1) that the stretch curves of various rings group themselves naturally into three categories. In this study, these groups are treated as units. The first, including the ascending aorta and arch, down to the first intercostal artery, hereafter called simply the arch, measured an average length of 55 mm. The second segment, which included all thoracic aorta to the diaphragm, was 155 mm. long, and the final segment, the abdominal aorta, 109 mm. Longitudinal shortening upon removal from the body averaged 33 per cent for the abdominal region and arch, and 23 per cent for the thoracic. Stretch figures developed from the relaxed segments were corrected to the *in situ* lengths by these factors. The average volume-pressure relations so developed are shown in table 1.

In table 2 are given transmission times from the heart to the fork of the

[1] The expenses of this investigation were partly defrayed by a grant from the American Medical Association.

iliacs, as actually measured in living animals when the diastolic pressure was varied through injections of epinephrin or acetylcholine. Agreement between such measured velocities and those calculated from tension-length curves given when tension was applied very rapidly (1) is good, so that the total transmission time has been apportioned to the various segments as the rapid stretch data would indicate.

TABLE 1

Average volume-pressure relations of the parts of the 15 kgm. dog aorta

PRESSURE	ARCH	THORACIC	ABDOMINAL	TOTAL
mm. Hg	*cc.*	*cc.*	*cc.*	*cc.*
0	4.56	7.70	3.89	16.1
20	5.90	8.42	4.43	18.7
40	7.52	9.38	5.08	22.08
60	9.66	10.67	5.80	26.1
80	12.10	12.46	6.53	31.1
100	14.14	14.34	7.11	35.6
120	15.90	16.15	7.69	39.7
140	17.21	17.72	8.18	43.1
160	18.25	19.08	8.53	45.9
180	19.00	19.87	8.72	47.6
200	19.68	20.30	8.95	48.9

TABLE 2

Average transmission times, in milliseconds, through the various aortic regions of the dog

PRESSURE	ARCH	THORACIC	ABDOMINAL	TOTAL
mm. Hg	*m. sec.*	*m. sec.*	*m. sec.*	*m. sec.*
20	18.3	58.8	27.2	104.3
40	14.8	47.6	23.2	85.6
60	12.3	39.2	19.7	71.2
80	10.4	31.9	17.3	59.6
100	9.0	26.8	15.2	51.0
120	7.8	23.4	13.6	44.8
140	6.8	20.3	11.9	39.0
160	6.0	17.0	10.6	33.6
180	5.1	13.8	9.2	28.1

To approximate, by calculation from the pressure pulse contour, the time course of ejection, it was assumed that the central pulse contour passed unchanged through the aorta, and out the aortic branches to the arterioles. The contour of the actual pulse which is seen in the peripheral arteries, of course, does differ from that seen in the upper aorta. The difference is largely due to the superimposition of reflected waves from the arterioles (5, 6), which in turn are due to arteriolar resistance to flow. The pressure rise attendant upon reflection is additional to that caused by the cardiac ejection, and is therefore not considered in calculating the time course of ejection.

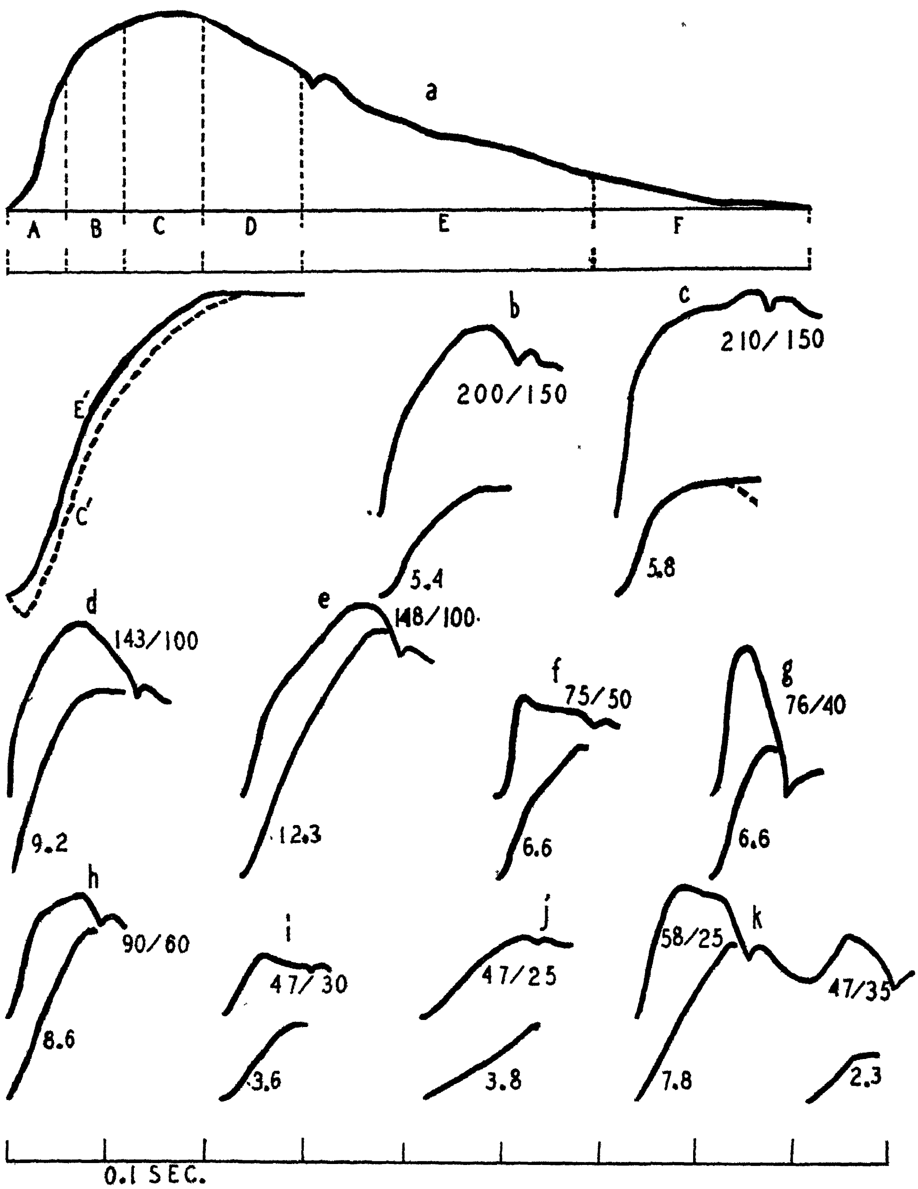

Fig. 1. Cardiac ejection curves as derived from pressure pulse contours for the dog. a. Central pressure pulse from Wiggers and Katz (7), divided into areas for the estimation of drainage (see text). Curve E', the corresponding derived ejection curve, and curve C', the corresponding cardiometer curve from Wiggers and Katz. *b* and *c* are pulses following the injection of epinephrin. Systolic and diastolic pressure values, in millimeters Hg, are given. Adjacent to the ejection curves are the total stroke volumes, in cubic centimeters. Curves *d* and *e* are normal pulses, curves *f*-*k*, a variety of low pressure pulses. Curves *f*, *h* and *i* were taken following the injection of acetylcholine. The others were from animals in shock. Curve *k* shows an alternating heart.

To illustrate the method employed in the construction of a cardiac ejection curve, a central pulse contour has been taken from Wiggers and Katz (7) (fig. 2, second cycle), and given arbitrary diastolic and pulse pressure values of 80 and 48 mm. Hg respectively (fig. 1). The weight of the animal in question was assumed to be the same as our average (15 kgm.). The pulse was now divided into 10 m.sec. units, with the average pressure above diastolic level for each unit recorded. Our hypothetical aorta is also divided into units whose length shall represent 10 m.sec. transmission time, and from the data given in table 1, the volume increment for each pressure rise, per 10 m.sec. length of tube, has been calculated. The calculation is given in table 3. During the first 10 m.sec., the wave has traversed the first aortic segment, and the pressure therein has

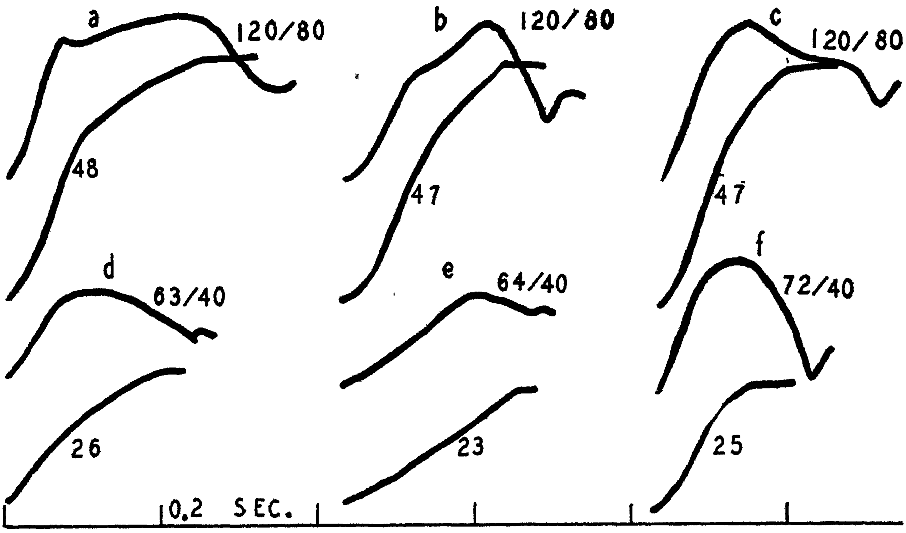

Fig. 2. Cardiac ejection curves derived from axillary and carotid pulse contours for the human. The systolic and diastolic pressure values, in millimeters Hg, are adjacent to each pressure curve; the derived total ejection volume, in cubic centimeters, to each ejection curve. Curves *a*, *b* and *c* are normal pulses. Curves *d*, *e* and *f* were taken from patients in shock.

risen 3 mm. Hg. This means that 0.31 cc. has been ejected into it. During the second 10 m.sec. period, the first segment has an average pressure of 5 mm. Hg above diastolic, its blood content has increased to 0.51 cc. above diastolic, and the pulse wave has traversed the second aortic segment. Here the pressure has increased 3 mm. Hg and the blood content 0.09 cc. During the third 10 m.sec. interval the pulse wave has gone one segment further, and the first two segments have increased their blood content as indicated. Succeeding intervals are treated alike, and the total uptake summated during each interval in the last column. To save space, several intervals are omitted from the table. These summated volume changes will give an aortic uptake curve which will satisfy the pressure pulse contour.

During the ejection period, drainage from the aorta, through the arterial branches, has been occurring. It was assumed that the volume outflow is proportional to the pressure obtaining over 20 mm. Hg (8), and that the total outflow during diastole would equal in amount the aortic uptake. For simplified treatment, the pressure curve was divided into several regions whose respective areas above 20 mm. Hg could be calculated as though they were trapezoids (fig. 1). The sum of areas E and F, which should represent the diastolic drainage, was regarded as equivalent to the volume of the aortic uptake. The drainage volume representative of regions A, B, C and D was then calculated from the proportion of their respective areas to the summated area of E and F

TABLE 3

The construction of an aortic uptake curve for the dog aorta

TIME INTERVAL	* PRESSURE RISE	VOLUME UPTAKE, CC.							
		ARCH	Thoracic				Abdominal		Total
m.sec.	*mm. Hg*	*10 m.sec.*	*32 m.sec.*				*17 m.sec.*		
10	3	0.31							0.31
20	5	0.51	0.09						0.60
30	12	1.22	0.15	0.09					1.46
40	19	1.94	0.35	0.15	0.09				2.53
50	26	2.57	0.56	0.35	0.15	0.02	0.04		3.69
60	33	3.18	0.76	0.56	0.35	0.03	0.07	0.04	4.99
70	37	3.58	0.96	0.76	0.56	0.07	0.17	0.09	6.19
80	40	3.80	1.06	0.96	0.76	0.11	0.26	0.19	7.14
90	42	3.92	1.15	1.06	0.96	0.15	0.36	0.29	7.89
100	43	3.98	1.19	1.15	1.06	0.19	0.46	0.39	8.42
120	46	4.19	1.27	1.23	1.19	0.23	0.57	0.56	9.24
160	48	4.32	1.33	1.32	1.31	0.26	0.65	0.67	9.86
200	47	4.26	1.33	1.35	1.35	0.27	0.68	0.75	9.99
240	44	4.05	1.28	1.30	1.31	0.26	0.65	0.75	9.60
280	39	3.71	1.17	1.20	1.23	0.25	0.61	0.71	8.88
300	35	3.36	1.06	1.12	1.17	0.24	0.59	0.66	8.20

* This column applies only to arch. It should be lowered one space to apply to the next column and so on, beginning at the level at which each column begins.

(table 4). From these values, it is possible to calculate the volume outflow from the whole aorta for each 10 m.sec. time interval.

It was assumed that drainage loss from each general region of the aorta would be proportional to its reservoir capacity (but cf. 9). In other words, drainage would be from the nearby rather than from the distant regions of the aorta, and, during diastole, would involve little movement of blood up or down the aorta.

During diastole, drainage out the several aortic branches is at a rate that gradually decreases as the pressure falls off. During systole, as the pressure rises, as soon as the pulse wave arrives at each successive segment of aorta, blood will leave through the branches at an increased rate. At first, this blood

expands the arterial branches. Later, as the pulse wave reaches the terminal arterioles, drainage will increase as the pressure rises. Since it is practically impossible to evaluate the amount by which the many arteries expand, we have used the simple dodge of saying the outflow from the aorta bears the same relation to pressure whether the blood concerned goes to fill the branches or actually leaves the arterioles as drainage. An error is probably involved, but one which will not significantly affect the contour of the ejection curve.

Drainage from the lower parts of the aorta before the arrival of the pulse wave is not included in the calculations, since this drainage merely serves to bring the pressure in the lower aorta to the diastolic level, and hence belongs properly with the diastolic drainage of the previous cycle. On the basis of these considerations, the volume outflow from each 10 m.sec. segment of the aorta during each 10 m.sec. interval of time was calculated. A listing of these quantities, and their summation is given in table 5, which is again an abridgement of the actual work sheet. A shorter listing of drainage quantities is given

TABLE 4

The estimation of drainage from the dog aorta

PULSE REGION	AVE. PRESSURE HEIGHT	TIME LENGTH	AREA	VOLUME
	mm. Hg	*sec.*		
A	78	0.06	4.68	1.19
B	102	0.06	6.12	1.55
C	107	0.08	8.56	2.17
D	101	0.10	10.10	2.57
			29.46	7.48
E	82	0.30	24.60	
F	64.5	0.23	14.84	9.99
			39.44	

for the lower than for the upper parts of the aorta because the incisura occurs, and ejection ceases, 300 m.sec. after the beginning of systole. All drainage which occurs after this instant is at the expense of blood which has already been ejected and hence does not modify the ejection curve.

The aortic uptake (table 3, column 10) and the systolic drainage (table 5, column 9) were added to make the cardiac ejection curve which is given in figure 1 (curve E'). If the total volume change of the cardiometer curve of Wiggers and Katz (7), which corresponds to the pressure curve taken as the basis of calculation, is assumed to be the same 15.3 cc., the volume change at the respective time intervals will be shown in figure 1 (curve C'). The agreement with the calculated curve is rather surprising. The only major discrepancy is in the initial "ear" of the cardiometer curve, and a time lag that decreases as the curves meet at the top. This is explained in part as the result of movement of the heart into the cardiometer during early systole, and its movement out again in late systole (7).

Systole on this pressure curve lasts 300 m.sec., as measured from the begin-

ning of the pressure rise to the beginning of the sharp downstroke of the incisura. Ejection, however, lasts only 280 m.sec. This duration is the same on both the cardiometer curve and the calculated curve. Calculations continued beyond this time indicate that the aorta is losing volume at a rate greater than can be accounted for by drainage at the pressures obtaining in the various parts, on the hypothesis that a root pressure pulse is propagated unchanged down the aorta. The mean pressure producing drainage during late systole may be greater than this in the lower parts of the aorta, due to reflected waves. Also, we have shown (1) that a given pulse contour does not pass unchanged through the aorta, because of a viscous resistance of the aortic wall to the first part of a stretch. Whatever the cause of the seeming decrease in aortic content (plus drainage) in late systole, it is of small measure and certainly should not be recorded as a diminution in the height of the ejection curve (dotted line, fig. *c*). Such a decrease would imply that blood flows back into the heart, which is impossible since the aortic valves would prevent such a regurgitation. The top of the curve is therefore drawn flat to agree with the cardiometer curve, and to imply that in late systole ejection has ceased. In this period, the ventricle continues to maintain its contraction, and hold the intraventricular pressure equal to aortic, until such time as ventricular relaxation occurs and the valves close.

In the light of the agreement between the contour of the cardiometer curve and that of the calculated ejection curve, it seems worth while to discuss briefly the nature of the curves derived from some central pulse contours, taken when the chest of the dog was closed, and under varying physiological conditions. It may be expected that the extent and contour of the ejection curve would be affected by such factors as the length of systole, the height of diastolic pressure, the height of the pulse pressure, and the degree of "fullness" of the pressure pulse.

The duration of systole may vary, in different experiments, by twofold or more. Our own experiments with closed chest show a systolic duration of 120–170 m.sec. for normal pulses, whereas with the rapid heart and low diastolic pressure of the failing animal, this time may be reduced to some 60 m.sec. These values are in contrast to the 300 m.sec. duration of the Wiggers and Katz curve. The difference could easily lie in a cooling of the heart by exposure and an interference of coronary circulation by the cardiometer in these open chest experiments. There are records from this laboratory, in dogs with open chests, which show a systolic duration of 200–300 m.sec.

Other things being equal, the longer the systole, the greater will be the ejection. The stroke volume calculated from the Wiggers and Katz curve is almost twice that derived from one of our records with the same pulse pressure, and with systolic time roughly half as long. This volume difference is more apparent than real. We have assumed that the characteristics of the aorta of the Wiggers dog were those of our isolated aortas. Such an assumption may easily be less justified in an open chest animal than in a normal one, and hence physiological conclusions can hardly be based upon the size of the stroke volume so determined.

Smooth muscle tone changes may alter the diastolic volume of the aorta in considerable measure (2). Pulse wave velocity in the isolated aorta at various diastolic pressures is within the normal limits of variation values determined for the intact animal (1). In substance, then, it would seem that the proportion by which the aorta is distended by the pressure pulse is the same whether the aorta be dead and contracted, dead and relaxed, or alive and contracted to an unknown degree. The absolute volume of the aortic uptake, on the other hand, depends upon the diastolic capacity of the aorta. If we should assume that the aorta in the Wiggers dog was contracted to the degree that it contained only one-half the volume of the equivalent dead aorta, the stroke volume would be a half that expressed in figure 1. Further, we have arbitrarily given this pulse a pressure height of 48 mm. Hg, which is larger than might be expected from an animal with open chest. Whatever absolute value of the stroke volume be taken as physiologically valid, the agreement between the cardiometer and

TABLE 5

The estimation of systolic drainage for the dog aorta

TIME PERIOD	DRAINAGE VOLUME, CC.								
	ARCH	Thoracic				Abdominal		Total
m.sec.								
20	0.13	0.02						0.15
40	0.28	0.07	0.04	0.02				0.41
60	0.48	0.11	0.09	0.07	0.00	0.02	0.01	0.78
80	0.68	0.18	0.15	0.11	0.02	0.07	0.05	1.26
100	0.88	0.26	0.22	0.18	0.03	0.10	0.08	1.75
150	1.38	0.40	0.31	0.34	0.06	0.20	0.18	2.93
200	1.88	0.56	0.53	0.50	0.10	0.30	0.28	4.15
280	2.68	0.80	0.77	0.73	0.14	0.45	0.42	5.99
300	2.88	0.86	0.83	0.84	0.15	0.50	0.48	6.50

derived curves would be unchanged since it is an agreement of contour rather than quantity. It should be emphasized again, therefore, that differences in stroke volume obtained in various ejection curves is insecure. Differences in contour which correspond to differences in the form of the pressure pulse do, however, have a valid basis, and are significant. Some of these contours, developed for a variety of pulse curves, are given in figure 1.

The systolic portion of the pressure pulse usually has a pronounced shoulder, at some 15–30 m.sec. after the first pressure rise, after which the pressure rises less rapidly. This shoulder cannot be attributed to an inflection of the cardiac ejection curve, but rather to an increase in the length of the aorta expanding and draining under the influence of continued injection. If the ejection rate were constant, this time interval to shoulder should bear a constant relation to the transmission time. Actually, despite differences in ejection rates and variations in pulse wave velocity, this time interval varies only by a few m.sec.

After the shoulder, the pressure pulse may remain flat (fig. 1, *f*, *i*), may ascend to an early peak (*d*, *g*, *k*), to a peak in mid-systole (*h*), or in late systole (*b*, *c*, *e*,)*j*.

Beneath each pulse is an ejection curve calculated in the manner described above. Often ejection continues after the pressure begins to fall, because more distant reaches of the aorta are accepting blood and because drainage is augmenting. This is most typical of pulses arising from a low diastolic pressure level, at which the pulse wave velocity is greatly decreased. In general, early peaks in the pressure pulse are associated with a rapid rise and early flattening of the ejection curve, late peaks with the continued, slower ejection throughout systole.

A factor which proves troublesome in calculating the ejection curve is the return of the reflected wave to the root of the aorta before a prolonged systole is over. This happens when the transmission time is shortened by a high diastolic pressure. An example is shown in figure 1, *c*, which is a pulse contour obtained after the injection of epinephrin. Usually systole is over before the reflected wave returns. Where it is evident from the transmission times and from the contour of the central pulse that the reflected wave has returned before the incisura, an estimate must be made of the true pressure level, without the component to be related to the returning wave, for the calculations.

Changes in diastolic and pulse pressure. Other things being equal, it is obvious that the greater the pulse pressure, and the less the diastolic pressure, the greater will be the stroke volume. Thus curves *b* and *c* (fig. 1) have smaller calculated stroke volumes than *d* and *e*, even though the pulse pressure is of the same order, because the diastolic pressure is higher. The greater pulse pressure of *e* renders a greater stroke volume than for *d*, where the diastolic pressure is the same. Again the point should be stressed that differences in stroke volume inferred from differences in pulse pressure are not necessarily valid, since nothing is known about possible changes in diastolic size of the aorta. Since the tone of the aortic wall cannot change in the course of a single systole, differences in contour of the ejection curves, in different pressure ranges, is significant. This is illustrated by the comparison of curves *c* and *f* (fig. 1), where the pressure contours are similar, but *c* is over a higher range than *f*. In *b*, ejection flattens off as the aorta becomes less distensible at the high pressure levels, while in *f*, ejection continued unabated until very late in systole.

Human pressure pulses lend themselves to the same type of calculation, assuming that the carotid or axillary pressure pulse is representative of the central contour. Measurements of aortic cross area as given by Bazett *et al.* (10) are of a smaller order than those given by Hallock and Benson (11). To secure the relationships tentatively for the human aorta, the following measurements were made on an aorta from a negro woman 18 years old, height 167 cm.; weight, 50 kgm., surface area 1.55 sq. m. The *in situ* length of the ascending aorta from heart to innominate artery, was 6.3 cm.; from innominate to first intercostal, 5.9 cm., from first intercostal to diaphragm, 18.6 cm., and from diaphragm to fork of the iliacs, 11.0 cm. The aorta was cut into rings, and stretch curves developed at various rates of tension increment. As with the dog aortas, this aorta was divided into three regions, and volume-pressure relations developed for each part (table 6). On the basis of rapid stretch curves, transmission times

through the various parts were estimated, assuming the relationship true for the dog to hold for the human (table 7).

Using various pressure pulse contours from recordings from human carotid or axillary arteries, ejection curves were calculated from the measurements of the human aorta, as shown in figure 2. Pulses *a*, *b* and *c* all have the same pulse pressure and diastolic pressure level, differing in the general form of the pressure curve. The derived stroke volumes are of comparable size, but the contours

TABLE 6

Volume-pressure relations for a human aorta

PRESSURE	VOLUME			
	ARCH	Thoracic	Abdominal	Total
mm. Hg	*cc.*	*cc.*	*cc.*	*cc.*
0	25.8	19.0	7.4	52.2
20	33.4	21.8	9.1	64.3
40	44.0	26.0	11.3	81.3
60	56.8	31.5	13.8	102.1
80	75.0	38.6	16.4	130.0
100	85.3	46.4	19.0	150.7
120	90.2	52.6	20.3	163.1
140	95.6	57.0	21.3	173.9
160	98.0	59.6	22.3	179.9
180	99.5	61.3	22.8	183.6

TABLE 7

Estimated transmission times through a human aorta

PRESSURE	TRANSMISSION TIME			
	Arch	Thoracic	Abdominal	Total
mm. Hg	*m.sec.*	*m.sec.*	*m.sec*	*m.sec.*
20	36.0	54.0	32.0	122
40	31.4	48.0	27.7	107
60	26.8	42.0	23.2	92
80	22.4	36.0	18.7	77
100	17.8	29.7	14.4	62
120	13.5	23.0	11.5	48
140	10.7	18.0	9.7	38
160	8.4	13.5	9.2	31
180	6.3	10.6	8.7	26

are different. The same relations observed between pulse form and the course of the ejection curve in the dog, hold for the human pulses. Curves *d*, *e* and *f* represent three low pressure pulses, again with narrow pulse pressures. Pulse *f* is one of an oligemic form of shock. Despite the fact that the pulse pressure here is the greatest of the three, the short systole and rapid pressure decline curtail the ejection volume. Pulse *e*, on the other hand, is from a shock of neurogenic origin, and reflects a slowly rising but prolonged ejection.

SUMMARY

On the basis of the pressure pulse contour, the distensibility of the aorta at different levels, and the pulse wave transmission time, the form of the ventricular ejection curve has been calculated for the dog and for man. Possible changes in aortic tone make insecure the stroke volumes derived from these curves, but should not influence their contours, which are found to agree fairly closely with cardiometer curves published in the literature. Such derivations show that the course of the ejection curve may vary markedly when the pulse pressure and total stroke volume remain the same.

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THE RELATIONSHIP BETWEEN THE CARDIAC EJECTION CURVE AND THE BALLISTOCARDIOGRAPHIC FORCES[1]

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The movements which the human body makes in response to the impacts of the heart and blood have been studied occasionally since 1877 (1–5). The application of modern methods of optical recording enabled Starr and his co-workers to analyze these body movements, and to make a quantitative reconstruction of internal forces which could give origin to such deflections (6–10). The ballistocardiogram, as it is called, records a headward movement of the body (H wave), followed by a strong footward movement (I wave). This is terminated and followed by a strong headward movement (J wave), after which there are several waves (K, L, M, N, O) which may or may not die out completely before a second heartbeat. These later waves Starr regards as due to after-vibrations, produced by a quivering of the jelly-like body after the main impacts have acted.

Starr's calculations relate the size of the stroke volume to the size of the recorded I and J waves. For, from his analysis, the I wave is a reflection of the cardiac recoil from accelerating the blood upward into the aorta and pulmonary arteries. The J wave is the resultant of three forces: 1, that due to the deceleration of the stream in the heart, ascending aorta and pulmonary artery; 2, the impact of blood upon the crown of the arch; and 3, the acceleration of blood down the descending aorta.

Starr's analysis is based on the assumption that the velocity curve recorded by Machella (11) depicts a pattern which is characteristic of the velocities developed in the aorta of man, and that this pattern does not vary in its time course whether the stroke volume be large or small. The Machella curve was obtained by the hot wire method. The dog's chest was open and its heart rate was very rapid (over 240 per min.). Since the arterial pressure pulse was not recorded and since velocity ordinates were not given, one may question whether the curve was published as an example of the application of the hot wire method to a difficult situation rather than as a description of the aortic velocity curve of normal man. The fact that the I wave does not begin until the pulse wave reaches the lower carotid artery first led us to doubt the advisability of deriving the ballistocardiogram from a velocity curve that resembles one taken under the conditions that obtained during the Machella experiment.

We have shown elsewhere (12) that by summating the uptake of successive segments of the aorta as a given pressure pulse wave passes out, and adding a volume for drainage, a cardiac ejection curve can be calculated which agrees

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in pattern with the curve traced simultaneously by a cardiometer. Both the cardiometer curve and the ejection curve derived from the pressure pulse contour would produce velocity characteristics altogether different from those of the Machella curve as interpreted by Starr (fig. 1, *a*). Moreover it is possible to reverse the procedure of calculation and derive a pressure pulse from the Machella

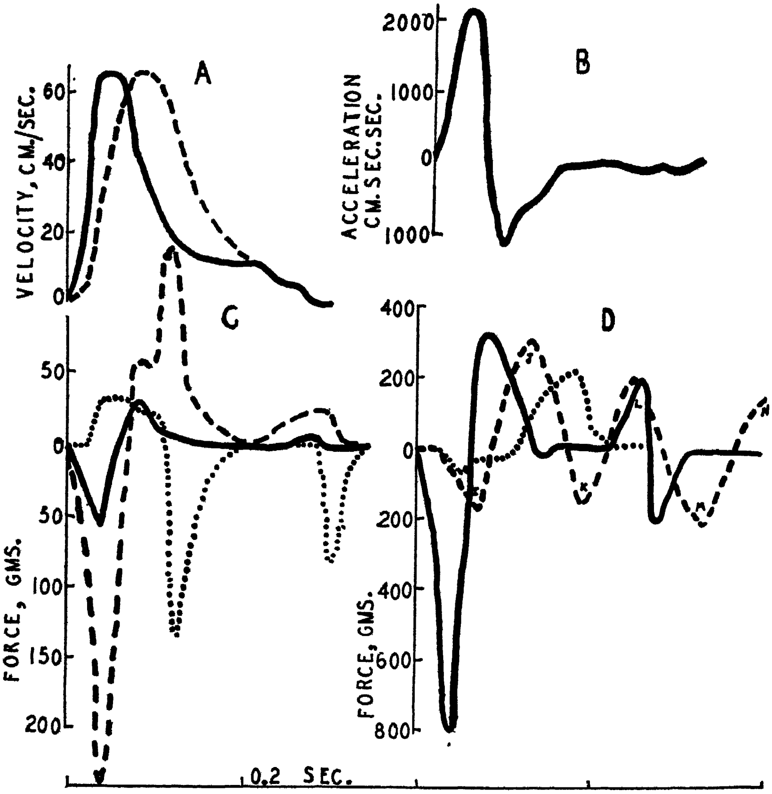

Fig. 1. The construction of a theoretical force picture for the human ballistocardiogram. *a*. Solid line, a velocity curve based on a theoretical human ejection curve (12). Broken line, a Machella velocity curve, as redrawn by Starr (7) to linear co-ordinates. *b*. The acceleration curve corresponding to the velocity curve given as the solid line in *a*. *c*. Derived force curves: Solid line, the cardiac force attendant upon ejection. Broken line, the force representing movement of blood in the ascending aorta. Dotted line, the summated forces representing movement of blood in the three segments of descending aorta. *d*. Solid line, the summated derived force values. Broken line, the ballistocardiogram given by a high frequency ballistocardiograph (7). Dotted line, the corresponding record from a low frequency ballistocardiograph (17).

velocity curve. Since the data were not given, we had to assign, arbitrarily, diastolic pressure and velocity ordinates to the Machella curve. We chose a relatively low diastolic pressure (50 mm. Hg) and maximum velocity (40 cm. per sec.) which we have found to obtain in experiments involving considerable trauma and a very rapid heart. When these figures are used, the Machella

curve gives a pressure pulse contour that shows a slow pressure rise and rounded peak typical of a shock condition. But when normal figures for diastolic pressure and maximum aortic velocity are assigned, the calculated pressure contour is quite beyond our experience.

We have presented elsewhere (12, fig. 2, *a*) an ejection curve derived from a normal human pressure pulse contour, with pressure values of 120/80 mm. Hg, and from the stretch measurements of a normal aorta taken from an undersized young woman. The stroke volume which is derived for this aorta is only 49 cc., in contrast to the 60 cc. which would be expected from the Grollman method (13), and the 80 cc. which we consider a more probable value (14, 15). Postmortem changes in the aorta may have restricted the aortic uptake and hence the total stroke volume to a figure that is less than would have obtained during life.

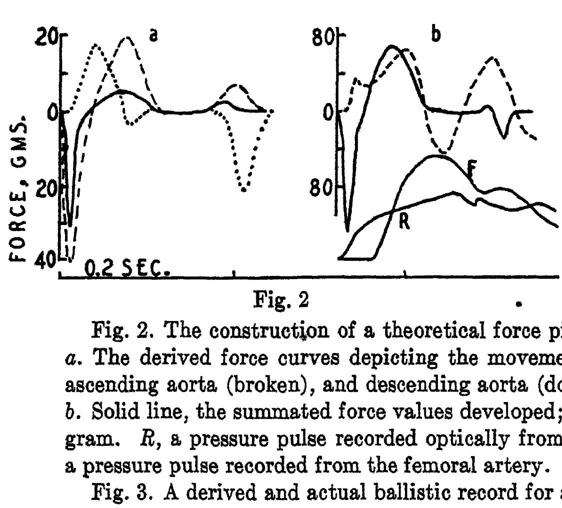

Fig. 2

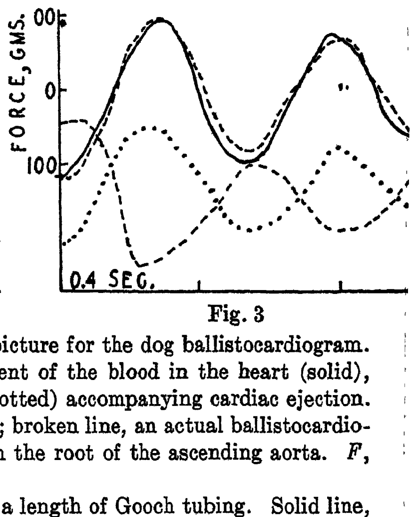

Fig. 3

Fig. 2. The construction of a theoretical force picture for the dog ballistocardiogram. *a*. The derived force curves depicting the movement of the blood in the heart (solid), ascending aorta (broken), and descending aorta (dotted) accompanying cardiac ejection. *b*. Solid line, the summated force values developed; broken line, an actual ballistocardiogram. *R*, a pressure pulse recorded optically from the root of the ascending aorta. *F*, a pressure pulse recorded from the femoral artery.

Fig. 3. A derived and actual ballistic record for a length of Gooch tubing. Solid line, the derived force curve. Broken line, the recorded force curve. *P* and *P′* represent pressure changes at the two ends of the tube, as recorded by optical manometers.

However, it has been shown elsewhere (12) that while such factors affect the amplitude of the ejection curve, they do not affect its time course.

Working on the assumption that the derived ejection curve has a physiological reality in form, if not in total volume, we have attempted a construction of the cardiovascular forces attendant upon the ejection of blood and the propagation of the pulse wave through the aorta, which might give rise to the movements of the ballistocardiogram. The great complexity of the situation renders inadequate any simplified description of the energy changes. Many features must be taken on faith, others must be assumed. The scheme we have used in this construction differs in some details from that used by Starr, but it seems more rational to us. It should be emphasized, however, that we make no pretense of assembling a strictly quantitative measure of the forces involved. Our only hope is that the picture as developed will offer a clue to the timing of

the various recoil movements, and also to the modifications in the ballistocardiogram which would accompany a change in the form of the ejection curve. As a first step, the velocity of blood leaving the heart in cm./sec. (table 1, column 4, fig. 1, *a*) was obtained by dividing the volume ejected during each 20 m.sec. interval, expressed as cc./sec. (table 1, column 2) by the changing cross sectional area of the orifice of the aorta (table 1, column 3). The table represents an abridged work-sheet with various steps eliminated for sake of brevity. Figure 1, *a* also shows a Machella velocity curve, as redrawn to linear coordinates by Starr, and given arbitrarily the same maximum velocity.

The average velocity change over each 20 m.sec. time interval was now plotted as an acceleration curve (fig. 1, *b*, table 1, column 5). To obtain a force curve reflecting this ejection, the acceleration values must be multiplied by a mass factor, and divided by 980. On the basis of several rather detailed analyses,

TABLE 1

The determination of the cardiac forces attendant upon ejection

TIME INTERVAL	EJECTION VOLUME	CROSS SECTIONAL AREA	VELOCITY	ACCELERATION	FORCE*
m.sec.	*cc./sec.*	*cm.*^{2}	*cm./sec.*	*cm./sec./sec.*	*gms.*
20	130	6.20	21	−1050	−26
40	450	6.90	65	−2200	−55
60	470	7.34	64	+50	+1
80	300	7.45	40	+1200	+30
100	270	7.60	30	+500	+13
140	110	7.70	14	+150	+4
180	95	7.80	12	+50	+1
240	50	7.65	7	+100	+2
280	0	7.50	0	+250	+7

* The product of acceleration (col. 5) /2 and a constant mass of 50 grams (see text).

we believe a fair approximation to this force may be obtained if we assume that the whole of the stroke volume, contained within the heart, is accelerated from zero to half the ejection velocity. Hence the mass involved is held constant at 48 (stroke volume) × 1.05 or 50 grams. The force curve obtained (table 1, column 6) is given as the solid line of figure 1, *c*. Using the convention of Starr, a footward recoil is given a negative sign, and plotted below the base line.

Now the blood which has been ejected will follow either of two courses. It may go to distend the ascending aorta, in which case it will lose its longitudinal velocity, or it may displace a portion of the blood already contained in that part of the aorta. The forces producing these movements of blood should be marked by recoils to which the ballistocardiograph is sensitive. To assist in their formulation, the aorta has been divided into four general regions, such that each section will be traversed by the pulse wave in 20 m.sec. The fact that at a diastolic pressure of 80 mm. Hg the total transmission time is 80 m.sec. makes these four segments include the whole of the vessel from the heart to the

bifurcation of the iliacs. The first of these segments includes the ascending aorta and crown of the arch, the other three comprise the descending aorta.

From the data upon which the ejection curve was assembled (12), it is a simple matter to calculate the velocity of blood entering and also leaving each segment at any given time. These two values are then averaged to give a mean velocity through the whole of the segment.

The deceleration of blood which will distend each of the four segments will be reflected in four monophasic curves. In section one, this force will be headward, in the others, footward. These force values have been obtained by multiplying the mass of the blood retained in the respective sections by the deceleration from the entering velocity to zero.

For the force depicting movement of the blood through the tube, the mean acceleration value for the whole segment has been multiplied by the whole mass of the contained blood. The two force values so developed for each of

TABLE 2

The determination of forces attendant upon the movement of blood through the ascending aorta

TIME INTERVAL	VOLUME ENTERING	VOLUME RETAINED	FORCE OF DECELERATION OF RETAINED BLOOD	MEAN VELOCITY	ACCELERATION, THROUGH BLOOD	MASS	FORCE
m.sec.	*cc.*	*cc.*	*gms.*	*cm./sec.*	*cm./sec./sec.*	*gms.*	*gms.*
20	2.6	2.6	+3	11	−550	84	−47
40	9.0	4.8	+17	63	−2600	90	−242
60	9.4	4.6	+16	81	−900	95	−83
80	6.0	0	0	69	+600	95	+58
100	5.4	0.1	+2	59	+500	95	+48
120	2.5	0.2		30	+1450	95	+140
140	2.2	0.2		23	+350	95	+34
180	1.8	0.2		18	+50	96	+5
240	0.9	−0.1		11	+150	95	+14
280	0	−1.0		3	+250	93	+24

the segments have been summated. In table 2 are given the calculations for the first segment of aorta with the final force values plotted in figure 1, *c* (– – –). The other three segments were treated in similar fashion, but all three forces were added to make a single curve for plotting purposes (fig. 1, *c*) (. . .).

The various force pictures are now complete. In their summation, the forces attendant upon the ejection of blood from the heart should be multiplied by two, so as to include both ventricles. Forces developed in the pulmonary circuit must also be taken into account. The pulse wave velocity in the pulmonary arterial system is said to be similar to that of the first part of the aorta 16). The cross-sectional areas of the two vessels are similar, while the ascending portion of the pulmonary artery is about $\frac{2}{3}$ the length of the ascending aorta. The forces developed for the first section of the aorta have therefore been multiplied by $\frac{2}{3}$. The thrusts effected by the descending aorta probably have little counterpart in the bifurcate pulmonary system, where the later thrusts are in

several directions and mainly lateral. Only the aortic values have been used in the summation of the later thrusts. Lastly, to bring the force picture more in line with a normal, 80 cc. stroke volume, these values, determined on the basis of a stroke volume of 48 cc., have been corrected upwards by the ratio of 80/48. Such a corrected curve is shown in figure 1, *d*.

That our method of calculation may not be too greatly in error is seen from figure 3. A similar construction was made for a length of Gooch tubing, filled with water, which lay on a ballistocardiograph and in which a system of standing waves was generated. The expected and actual records are quite similar.

For comparative purposes, the force pattern recorded by means of a high frequency ballistocardiograph of the type used by various earlier workers (3–5) and described in detail by Starr, is plotted in figure 1, *d* as a broken line. The recorded force was taken to be that represented by the average of Starr's cases (7, table 1). A comparison can also be made with the forces required to impart velocity to the body which are recorded with a low frequency ballistocardiograph (1, 2, 17) (dotted line, fig. 1, *d*). The calculation for the latter was based on the electrically integrated velocity curve of Nickerson and Curtis (17), from which was derived an acceleration curve. By multiplying the weight of the body and bed by successive acceleration values, the force necessary to accelerate the body and bed was approximated. The force necessary to overcome the damping resistance was also added. This varied in proportion to velocity, and was approximated by determining the force which would cancel the momentum during the period of the bed. To this summated curve was added an almost negligible component representing the restoring force.

There are two major discrepancies between the calculated and the recorded force patterns, that of onset time and that of amplitude. The recorded force begins consistently later than the initial force calculated from cardiac recoil, and the extent of the recorded force is less than that calculated as necessary to move the blood. This discrepancy is greater with the low frequency apparatus than with the high frequency.

Initial lag. It is difficult to see why the initial deflection of the I wave should be delayed 20 m.sec. after the beginning of ejection, for even an imperfect recorder should start its first deflection without lag. One explanation which could be offered is that, dating, as we have, the time sequence from the carotid surface pulse, the estimated heart-carotid transmission time is in error. Dating from the apex beat, Bazett and associates (18) set the transmission time to the subclavian artery at 60 m.sec. On the basis of stretch measurements made upon our human aorta, this interval seems much too long. Our estimate would be that not more than 30 m.sec. should suffice to the carotid, and it is upon such a figure that the reconstruction has been made. The comparison of a large number of ballistocardiograms with simultaneous carotid pulse records has led to the conclusion that the I wave starts, on the average, 5 m.sec. before the carotid pulse. If no time lag is present, this would require a heart to carotid transmission time of 5 m.sec., or a net pulse wave velocity of the order of some 30M. per sec. or more.

Another explanation for the delay of the start of the initial downstroke might be sought in the possibility that a relatively slow recorder such as the ballistocardiograph-body system might introduce delay in the record. Against this possibility is the fact that when the period of the recorder is changed from 0.14 sec. to 14 sec. (17) there is no significant increase in the lag between the R wave of the electrocardiogram and the initial downstroke of the ballistocardiogram.

A further possibility is that the delay in the appearance of the initial downstroke is due to delay in the transmission of the stress through the body tissues. Against this are the results of experiments on models. When the recoil curve of the model to be described later is recorded by means of a ballistocardiograph, the downstroke is simultaneous with the beginning of ejection. This is true whether the ballistocardiograph be adjusted to a high or to a low frequency. Moreover, it is true if the model is laid against the belly of a human resting on the ballistocardiograph and in such a way that the forces generated in the model are transmitted through the soft tissues of his abdomen.

Since the delay in the first downstroke cannot be explained by the manner in which the forces are transmitted or recorded, it is suggested that an explanation be looked for within the heart itself. The walls of the heart move during ejection, and so does the venous blood. The preponderant systolic movement of the heart walls is downward (19) and some at least of the venous blood moves downward during ventricular systole. These movements would give an upward recoil of the ballistic record. We see no way of ascribing to these recoils a quantitatively accurate force value. This upward force should begin in isometric contraction and continue for an undetermined period in early systole, and may account in part for the H wave.

In the dog there are forces which play an analogous but more extensive role. The footward force upon ejection is more transient than in the human, so that the whole of the I wave, rather than merely its earlier parts, is cancelled. The ejection curve derived from the dog aorta and pictured elsewhere (12, fig. 1, *d*) was used in calculating a force curve in the manner outlined above. This force curve is shown in figure 3, *b*. Superimposed as a dotted line is the ballistocardiogram taken at the same time as the pressure pulse from which the ejection curve was calculated. The I wave is absent from the recoil curve, though the forces which should produce it are necessarily present. No explanation for the absence of the I wave in the dog and its delay in man comes to mind except on the basis of hypothesized canceling forces connected with the process of early ejection.

The discrepancy in amplitude between the recorded forces and those necessary to move the blood is to be accounted for initially by the presence of the canceling force which delays the cardiac recoil. In addition there are the effects of viscous friction and the overcoming of momentum. All stresses which are put upon the recorder must be applied at the expense of movement of the cardiovascular system in relation to the body, and of the body in relation to the bed. These movements are against viscous frictional resistance and involve loss of energy. The more slowly the force is developed, and the longer it is sustained, the more

closely will the recorded force approach what we believe to be the actual force. Hence the J wave shows better agreement than does the last of the I wave, and the J wave of the dog shows relatively more force than does the wave of the human. Since part, at least, of the J force is spent in overcoming the momentum of the I downswing, it is hard to defend the correctness of arbitrarily reducing the ballistocardiographic record by 40 per cent to compensate for overfling (7).

The situation is much the same in the case of the low frequency ballistocardiograph. It was held by Nickerson and Curtis that in the absence of a restraining force (stiff spring), the body would not be thrown into the vibrations which give the later waves of the ballistocardiogram. The validity of this approach is dependent on the notion that the later waves are passive after-vibrations. If, on the other hand, the later waves reflect forced movements occurring late in systole and in diastole, they will distort the later stages of the low frequency ballistocardiogram, and render its quantitation insecure. Several groups of data indicate rather strongly that forced movements do occur after the I-J complex is completed.

First, we constructed a low frequency ballistocardiograph by suspending a light bed by long chains, and partly damped its oscillations by means of vanes in oil. The relative movements between the bed and the floor were recorded as a diphasic curve similar to that recorded by Nickerson and Curtis (17, fig. 2, *a*) (fig. 6). The relative movements of the body and bed were recorded by fixing the recorder on the bed, and recording directly from the head. The usual high frequency pattern appeared, with waves I to O (fig. 6). Moving the body relative to the bed therefore required energy not recorded by the bed. In other words, the forces overlap within the period of the recorder. It is not surprising, then, that only a part of the forces generated can ever be recorded. A larger part of these forces is recorded by a high frequency ballistocardiograph than by the low frequency device, but neither can be regarded as a perfect recorder.

Secondly, a model of the heart and aorta was constructed of a wide-mouthed bottle and a length of Gooch tubing. Ejection was made by inflating a cloth-covered balloon with air, which displaced water in the bottle and forced it out into the Gooch aorta. The whole was enclosed in an air tight glass jar, and the time course of ejection recorded plethysmographically with an optical capsule. Pulsations could be recorded from the two ends and the middle of the "aorta".

The onset of ejection was accompanied by a downward thrust (fig. 5, *a*). Probably because deceleration from a nearly constant injection slope was gradual, an upward thrust during ejection cannot be segregated as a distinct entity. It is clear, however, that the timing of the later oscillations shows a definite relation to the pulsations in the aorta. When the aorta was straight, the period of the "standing wave" and that of the ballistic waves were identical.

In figure 5, *b* is shown a similar record made when the "aorta" was symmetrically arched. In this case, "downward" thrusts would be developed by pressure rises at either end of the tube, while "upward" thrusts would develop with a pressure rise at the top of the arch. Since pressure oscillations at the ends of the tube will be exactly reciprocal, and the forces will cancel each other

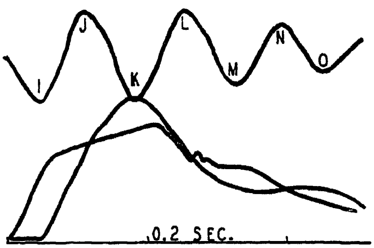

Fig. 4

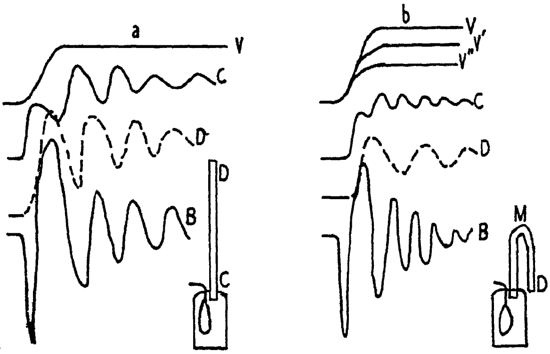

Fig. 5

Fig. 4. A correlation between the period of the waves of the human ballistocardiogram and that of the aortic standing wave system, as obtained from the carotid and femoral pulse contours.

Fig. 5. The ballistic record produced by the ejection of fluid in a model heart-aorta system. *a*. Records obtained from a straight "aorta", and *b*, from an arched "aorta". *V*—volume change; *c*—pressure oscillations at the central, orifice end of the tube; *M*—pressure change at the middle of the tube; *D*—pressure change at the distal end of the tube; *B*—the ballistic record.

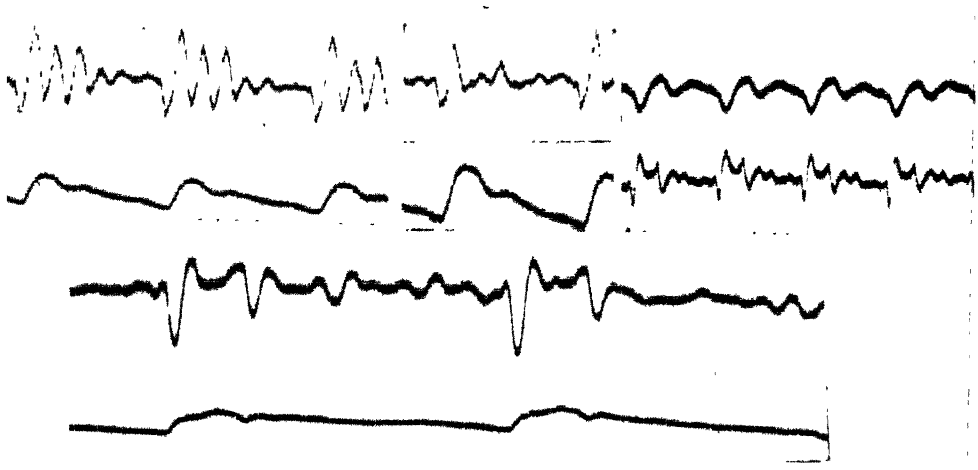

Fig. 6. Top left, a ballistocardiogram showing free oscillations, as though ejection and standing wave thrusts were in phase. Top center, a record from the same individual showing rapid damping, as though the two systems were out of phase (see text). The simultaneously recorded brachial surface pulse is given below the ballistocardiogram. Top right, a comparison between the records obtained from the low frequency ballistocardiograph and from the head of the individual (see text). Lower record, a ballistocardiogram, and corresponding carotid pulse record taken from a patient with aortic coarctation. Time marks represent $\frac{1}{12}$ sec.

the ballistic record now should follow only the pulsations in the arch, and have a frequency twice that of the standing wave. The record shows this to be true. It seems clear, therefore, that a well developed system of reciprocal pressure

oscillations at the ends of a tube can give rise to definite and predictable movements of the ballistocardiograph.

One could hardly expect to find such an exact agreement between the standing wave of the aortic tree and the later ballistocardiographic oscillations. As was shown by Starr's (7) experiments with cadavers, the viscous elastic mass of the body itself will show oscillations, after displacement, which have nearly the same frequency as that of the ballistocardiogram. Hence it is the body mass itself which, in large part, renders the ballistocardiograph but an imperfect recorder of rapid oscillations. The most that can be expected is that the pulsatile oscillations can modify the body oscillations. They cannot dominate them, and determine absolutely their timing.

Thirdly, the fact that the later waves of the ballistocardiogram do not damp out readily is evidence in favor of the view that a series of thrusts are being made in the later parts of the cardiac cycle. Damping was found to be quite rapid in the cadaver (7). After an impact was given to the body, each successive oscillation was 60 per cent as strong as the preceding one. In the ballistocardiogram, however, a series of two or three waves is often seen, in many successive cycles, in which there is little or no decrease in amplitude. On the other hand, sometimes the very opposite is seen, in which an established series of waves is abruptly brought to an end (fig. 6). It is as though the vascular and body oscillations were in phase and reinforced each other in some cases, and got out of phase and cancelled each other in other cases. In the case of the dog, the frequency of the pressure oscillations is so much greater than that of the body, that periodic waves are often not recorded by the ballistocardiogram at all. Even in the best of records, a correlation between the ballistocardiogram and standing wave appears only in diastole.

Fourthly, a definite correlation exists between the pressure oscillations seen on central and peripheral pulse records, and the later oscillations of the ballistocardiogram. In presenting this evidence, it might be well to describe briefly the formulation of the various ballistocardiographic waves, as we understand them on the basis of the reconstructions which we have attempted. The *H wave* occurs after most of the forces concerned with flow in the lower part of the body have died out. It begins with cardiac movements that take place during isometric contraction. The H wave is the most variable of the various waves, even from cycle to cycle. This is in large part due, we believe, to the variable amount of cancellation of a definite headward thrust from the heart, by the persistent, poorly defined, footward thrust which accompanies the transfer of blood to the peripheral portions of the lower body. In many cases, the H wave does little more than return the body to the original base line (as determined by equality of areas above and below, during a complete cycle). The *I wave* records the remainder of a partly cancelled footward thrust developed as blood is ejected from the heart into the ascending aorta and pulmonary artery. The delay is occasioned by the persistence of a headward force which began before ejection. The *J wave* has a complex origin. As we have calculated the forces, it registers the deceleration of blood in the heart, ascending aorta and

pulmonary artery, and an acceleration in the descending aorta. The forces concerned have been considered in detail above.

The *K wave* has not been considered in the analysis so far. It cannot be regarded as a simple afterfling of the body from the opposite thrust of the J wave for several very good reasons. First, it is often of greater magnitude than the J wave, which could not be true of a damped aftervibration. Second, the bottom of the K wave is, on the average, coincident with the peak of the femoral pulse curve (fig. 4). This coincidence is found when the standing wave period is long, as is the case when the subject is recumbent; also when the period is shortened by quickening the pulsewave velocity on assuming the erect posture (22) or by shortening the distance to the reflecting surface on occluding the arteries in the leg with a blood pressure cuff around the thighs. The standing wave period is shortened when the diastolic pressure rises. Starr (7, 8) and Page (23) have presented ballistocardiograms of hypertensive subjects which show a shorter than normal interval between the K and O waves. Records taken by them when the blood pressure was lowered through the use of depressor drugs (23), show a lengthening of the interval.

Since the femoral pressure pulse is composed of a summation of the propagated wave with its earlier parts which have been reflected from the arterioles in the lower part of the body, we can time, from its peak, the time of maximal reflection. If our concept is correct, then, this time should also be that of the peak of a strong footward thrust which will be recorded by the ballistocardiograph.

In the case of aortic coarctation, standing waves in the aorta do not develop. Blood pours asynchronously into the lower aorta through the collaterals, and the pulse pressure is less in the lower than in the upper aorta. The ballistocardiogram of such a patient shows little that is unusual in the I and J waves, but the K wave is very poorly developed (fig. 6). In this case it seems to be truly an afterswing. In many records it seems to be a plateau maintained above the rest level. It is certainly not a forced wave as is the normal K, which is simultaneous with the femoral peak (fig. 4).

The L and M waves can best be considered together. Blood in the ascending aorta decelerates at the end of systole giving a headward thrust (fig. 1, *c*, *d*). There may even be a reversal of the direction of flow, which would emphasize this thrust. The effect of this reversal of flow we have not attempted to quantitate. The headward thrust (L wave) will be cut short by a footward thrust as this reversed wave closes the aortic valves sharply. In addition, deceleration of blood in the descending aorta presents a footward thrust (fig. 1, *c*, *d*). Hence the peak of the L wave should be approximately timed to the incisura of the central pulse (fig. 4), and the M will follow the incisura by a variable interval. In aortic coarctation the M wave still persists as a strong footward thrust (fig. 6), even though deceleration of blood in the descending aorta would play little role. We can look upon the *N wave* only as an afterfling, for we have no internal force to which it can be attributed. Further, its appearance on the record does not suggest a definite thrust, as does, for example, the M wave.

The O wave is synchronous with the diastolic rise of the femoral pulse curve,

and is believed, therefore, to have its origin in the reflection of the pressure wave at the arterioles in the lower part of the body. Hence the O wave is analogous to the K wave (see fig. 4).

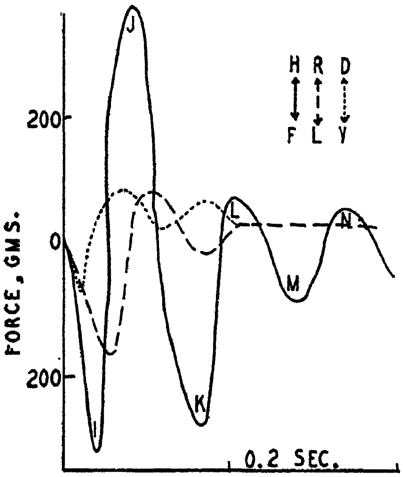

Fig. 7

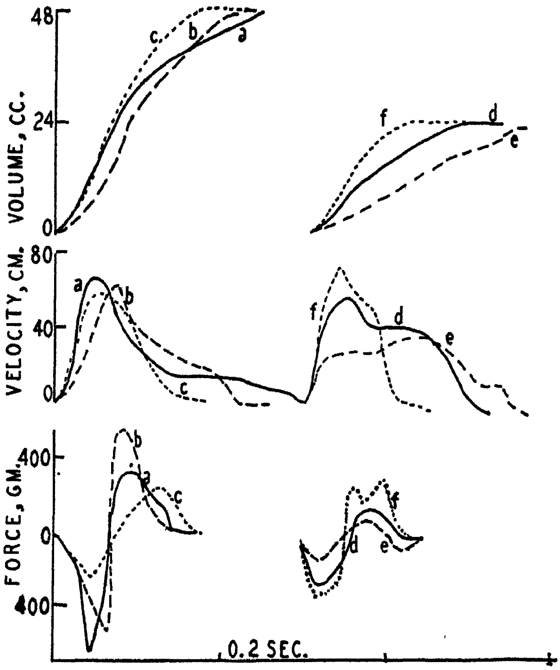

Fig. 8

Fig. 7. Simultaneous recordings from a three-directional ballistocardiograph.

Fig. 8. The influence of change in contour of the ejection curve upon the calculated force pattern. The ejection curves corresponding to three different normal pulse curves (*a*, *b*, *c*) and three curves from patients in shock (*d*, *e*, *f*) are given. Under these ejection curves are given the corresponding derived velocity curves, and the total forces which presumably will give rise to the ballistocardiogram. The areas under the derived force curves are as follows:

Curve	*Stroke Volume cc.*	*I Wave*	*J Wave*	*I and J*
a	48	235	481	416
b	47	240	212	452
c	47	105	169	274
d	26	125	101	226
e	23	52	31	83
f	25	161	157	318

Lastly, there is evidence of another sort that the forces which determine the later waves of the ballistocardiogram are related to the standing wave system. By three different simultaneously recording systems, we have followed the movement of the bed: *a*, headward and footward; *b*, right and left; *c*, dorsal and ventral. From a comparison of the records, several facts are apparent

(fig. 7). First, the direction of the force giving rise to the I wave is ventral, to the left, and footward, which is as might be expected from the position of the heart. The force giving the J wave is in the same axis, but headward. At some time past the peak of the J wave, the actual time being variable, the direction of movement changes, so that later waves are almost straight headward and footward, with but minor deflection to right or left, or dorsal or ventral. Hence two components seem to be involved. The first is related to the heart forces, the other, to a force effective along the long axis of the body, seemingly along the aorta.

From the above it appears that the forces involved in ejecting the blood are recorded with an unknown degree of diminution in the ballistocardiogram. It is next appropriate to find out whether these forces vary quantitatively with the stroke volume when the ejection curve is varied in form in such a manner as to produce the pressure pulse contours which are formed in ordinary experience.

The effect of changes in ejection contour upon the recoil curve was first investigated by means of the models described above. In the experiment shown in figure 5, *b*, the inflation was cut short so that the total ejection was reduced to $\frac{3}{4}$ or $\frac{1}{2}$ of the previous amount. This had no effect upon the recoil curve as long as ejection started off at the same rate. A smaller recoil curve could be had by initiating ejection more gradually and a larger one by initiating ejection more suddenly.

The variations in the human ejection contour greatly modify the relation between stroke volume and the ballistocardiographic forces. For example, in figure 8 (*a*, *b*, *c*) are shown three representative ejection curves for a human with diastolic pressure of 80 mm. Hg. The first (*a*), which was used to demonstrate the method of calculation (fig. 1), (12, fig. 2, *a*, *b*, *c*) corresponds to a central pulse which has an initial rapid pressure rise, a prominent "shoulder", and a pressure plateau until shortly before the incisura. Curve *b* is representative of a pulse which shows an almost constant pressure rise, reaching its peak very late in systole. Curve *c* is one with a pressure peak early in systole. Velocity curves derived from such ejection curves are, of course, quite different (fig. 8). Similarly, the calculated recoil forces are quite different. The areas under the I-J deflections as outlined show rather wide variations, although the stroke volumes of all three curves are the same.

Lastly, suppose we take three ejection curves representing three types of shock pressure pulses (12, fig. 2, *d*, *e*, *f*). Again the derived force picture differs appreciably for the three curves, and the magnitude of the calculated deflection changes while the stroke volume remains the same (fig. 8, *d*, *e*, *f*). In fact, the areas of I and J for curve f would indicate a stroke volume almost as great as for the normal pulses. That such variations in the contours of ejection affect the actual ballistocardiogram is indicated by the fact that Cournand *et al.* (14) have shown that the ballistocardiogram indicates a stroke volume that is altogether too large in some cases of shock.

SUMMARY AND CONCLUSIONS

The forces necessary to accelerate blood during systole were found to be greater than those recorded by the high frequency ballistocardiograph and to exceed still more those recorded by the low frequency instrument.

The later waves of the ballistocardiogram are forced movements and not merely afteroscillations of the body. The forces producing these forced movements are described in the text.

Ejection curves calculated from aortic uptake and drainage with normal and abnormal pressure pulse contours are as variable as the pulse contours themselves. The velocity, acceleration, and force curves differ widely in relation to the stroke volume.

Calculation of the cardiac output from the ballistocardiogram is therefore an empirical procedure and the values arrived at should be looked at as comparative rather than absolute. This does not deny the clinical value of the ballistocardiogram but indicates the need of an elaborate empirical research program to evaluate ballistocardiographic patterns in terms of disease and prognosis.

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ACQUIRED RESISTANCE TO WATER INTOXICATION[1]

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It is well known that animals and men can adapt themselves or develop a tolerance to a wide variety of stresses such as drugs, toxins, etc. Selye, in particular, has studied the nature of such adaptational processes and has stressed the features that are common to all of them (1). The observation has recently been added that animals can be adapted, by prior conditioning with sublethal stimuli, to withstand amounts of trauma which otherwise would produce lethal shock (2).

We had occasion to investigate the possibility that animals could develop a resistance to the toxic effects of excess water. It was found that they could do so as shown in the experiments described below. The only other recorded reference to this sort of phenomenon of which we are aware is that provided by a single sentence in Adolph's monograph (3): "Thus, repeated administration of water to a dog results in greater rates of urinary output at the same water excess (Kingsley)." We were apparently working in part with the same phenomenon here.

METHODS. Male rats approximating 175 grams in body weight were used. The methods were all the same as those used in previous experiments (4, 5). Distilled water warmed to body temperature was given in doses of 3 cc. per 100 sq. cm. of body surface to rats kept in individual metabolism cages. This dose amounts to 8 cc. for a 175 gram rat. The water was given through a stomach tube made of an 18 gauge hypodermic needle with a small bulb (2.3 mm. diam.) at the tip. An 18 hour fast preceded the tests. In diuresis experiments two or five doses of water were given at hour intervals; in intoxication experiments water was given at half-hour intervals until death or until 13 doses had been administered. Control and treated animals in approximately equal numbers, generally in groups of 6 each, were always tested simultaneously. Individual animals show a rather high variability (4) in experiments of this sort, but in any experiment in which significance is clear the average results of any one day's experiment will not differ qualitatively from those of another day, and when they do not so differ the results are referred to subsequently as "consistent." No significance is claimed for those apparent differences in over-all averages which were not seen consistently in successive experiments. It might be mentioned incidentally that the individual variability in such diuresis experiments would be much less apparent if the conventional method of grouping 4 animals to a cage had been used.

In those experiments in which 13 doses of water were used the statistical

[1] Aided by a grant from the Josiah Macy Jr. Foundation.

analysis was made on the figures obtained at the fifth hour rather than on those obtained terminally because at that time practically all animals were still alive. By the end of the experiment the number of animals was oftentimes greatly reduced particularly among controls, and the figures quoted represent in part a carry-over from previous hours.

The mortality figures include all animals dying within 24 hours of the end of an experiment. Body temperature figures were not obtained in all cases because suitable specially-made thermometers could not be obtained while some of the experiments were being conducted.

Technique for adaptation. To adapt animals to water one dose of water was given on the first day, two on the second, three on the third day, etc., until five doses were given on the fifth day. All of these adapting doses were given at half-hour intervals and without preliminary fasting. This treatment had little deleterious effect on the animals except that there was some decrease in the usual weight increment.

After the five doses of water were given on the fifth day, the animals were fasted the usual 18 hours, and on the sixth day given either two, five or thirteen doses of water and their responses compared with the two types of control animals described below.

No systematic attempt was made to determine if routines other than the one described would provide a more effective adaptation, but a few attempts to shorten the priming procedure indicated that less successful results were thus obtained.

Controls. Control animals were taken from stock cages at the same time as those adapted to water and all received identical care during the preliminary procedures. One type of control was simply a normal rat which had received no previous treatment. The second type consisted of rats in which a stomach tube had been inserted at the same time and at the same intervals that water had been given to the water-adapted animals.

Results. The most clear-cut results were obtained when the adapted animals were tested for their resistance to 13 doses of water. As shown in figure 1 and table 1, series 1, they were far superior to normal controls in the rate at which water was eliminated, in their maintenance of body temperature and in their survival. Since the extent and degree of a developing state of water intoxication is most reliably indicated by changes in body temperature (4), particular importance is attached to the relatively slight fall in adapted animals. Mortality figures are less consistent and less reliable in general but the differences were of unmistakable significance here. To casual observation other signs of water intoxication were minimal; the typical convulsions were seen, generally in mild form, in only 30 per cent of the cases whereas they occurred in over 90 per cent of the controls.

Rats which had been adapted to the passage of a stomach tube, but not to the receipt of water, showed a completely intermediate response in all respects. The differences in the amount of water excreted by normal and stomach tube-adapted animals at the fifth hour failed by a fraction to exceed twice the standard

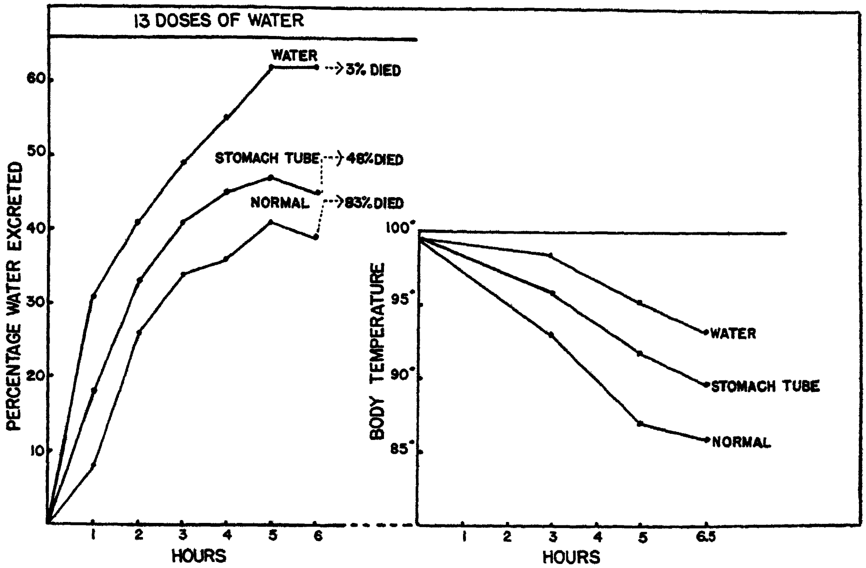

Fig. 1. Showing the rate of water excretion, mortality and changes in body temperature in rats given up to 13 doses of water at half-hour intervals. The average water dose was 8 cc. "Normal" refers to rats untreated previously; "Stomach Tube" to those adapted to receiving a stomach tube; "Water" to those adapted to receiving water by stomach tube. Additional data are given in table 1.

TABLE I

Effects of various treatments on response to water ingestion

SERIES NO.	TYPE OF ANIMAL	NO. RATS	PER CENT WATER EXCRETED AT FOLLOWING HOURS:				BODY TEMPERATURE (°F.) AT FOLLOWING HOURS:			PER CENT DIED	Cl. EXC.† (AS MGM. NaCl)
			3rd	5th*	6th	6.5	3rd	5th†	6.5†		
	Water-adapted and stomach tube adapted: Given 13 doses of water										
1	Water-adapted	30	49	62 ±2.9	62		98.5	95.3 ±0.67 (18)	93.5 (17)	3	49.6 (12)
	Stomach tube adapted	23	41	47 ±1.9	45		95.8	91.9 ±0.59 (17)	89.8 (15)	48	67.1 (12)
	Normal	23	34	41 ±2.5	39		93.2	87.8 ±0.58 (23)	87.8 (4)	83	61.0 (11)
	Water-adapted, vasopressin-treated: Given 5 doses of water‡										
2	Adapted + vasopressin	18	6	19				90.7 ±0.92		22	
	Normal + vasopressin	18	11	21				87.5 ±0.46		50	
	Water-adapted, adrenalectomized: Given 5 doses of water										
3	Adapted adrenalect.	17	42	37 ±3.5			98.4	95.9 ±0.97		6	
	Normal adrenalect.	19	36	31 ±3.7			97.5	93.9 ±0.89		0	
	Adapted to intraperitoneal glucose: Given 13 doses of water										
4	Glucose-adapted	16	41	45	47	52	95.2	90.4 ±0.93 (15)	88.7 (6)	50	52.5
	Normal	16	35	41	42	46	92.6	88.2 ±0.76 (13)	86.4 (3)	75	68.1

* Standard error included.

† Numbers in parentheses indicate number of animals on which determinations were made if less than total number of group.

‡ Normal rats given 5 doses of water show slight if any changes in body temperature and excrete from 65 to 70 per cent of the water given at the fifth hour (Gaunt, 1944).

error of the difference between the means; they were, however, consistent and appeared in the averages of every experiment. In any case the differences in body temperature were certainly significant. In addition the animals adapted to receipt of a stomach tube showed greater apparent well-being, fewer convulsions and smaller mortality than normal controls.

Small doses of water. When either two or five doses of water were given to the water-adapted and control animals the excretion rate was again in mean terms higher in the former. The differences did not appear in every experiment, however, and significance is not considered to be established.

Chloride excretion-table 1. Chloride excretion tended to be lower in water-adapted than in control animals. This was likely because part of the store of chloride was lost in the diuresis associated with the adapting procedure of the day before the test—a loss shown to be sufficient to reduce plasma levels slightly. Since salt depletion increases sensitivity to excess water this makes the greater resistance of water-adapted animals all the more impressive.

Resistance to water intoxication in vasopressin-treated rats—table 1, series 2. Attention has been called to the fact that the resistance to water intoxication is determined in part by factors other than variability in the diuretic rate (6, 7). This was particularly well illustrated when diuresis was inhibited in water-adapted and control animals by injections of pitressin tannate-in-oil[2] given simultaneously with the water.

Five doses of water were given at hour intervals since that is sufficient to produce toxic symptoms under these conditions. The effect of the vasopressor principle in inducing susceptibility to water intoxication had been long since demonstrated by Rowntree (16). Subcutaneous injections of 0.05 unit of pitressin (in 0.05 cc. of oil) were given with the first dose of water and repeated with the fourth dose.

The results show that the increased diuresis usually seen in adapted animals can be fully inhibited by vasopressin. Adapted and normal animals excreted the same amounts of water or, stated otherwise, retained the same amounts of water—a fact checked by body weight figures.

In addition, although similar amounts of water were retained, the adapted animals showed less marked signs of water intoxication than normal animals as judged again by general appearance, survival, and significant differences in body temperature.

Effect of adrenalectomy upon acquired resistance to water intoxication—table 1, series 3. Since it has been shown that the adrenal cortex is a factor of great importance in the maintenance of normal water diuresis and in resistance to the toxic effects of water and that a hyper-normal resistance can be produced by administering cortical hormones to intact animals (4), it is possible that the acquisition of resistance seen here may be mediated through increased activity of this gland. Our experiments are not conclusive on this point, but it is clear that the adaptation was largely if not entirely lost when the adrenals were removed.

[2] Pitressin tannate-in-oil was kindly supplied us by Dr. Oliver Kamm, of Parke, Davis & Company, Detroit.

Animals were adapted in the usual way, adrenalectomy then performed, and their response to five doses of water, an amount sufficient to produce toxic symptoms after adrenal ablation, determined 18 hours later. The rate of diuresis and the maintenance of temperature was in all series a little better in the adapted animals than in the non-adapted adrenalectomized controls. Statistical significance of the differences, however, was not clear. The suggestion of a positive result is reminiscent of the carrying-over of resistance to other stresses after adrenalectomy as reported by Selye (14).

Effect of adaptation to intraperitoneal glucose on resistance to water intoxication—table 1, series 4. The question arose as to whether the acquired resistance to water was specific, as seems to be true of the resistance to trauma (2), or whether it was a non-specific resistance to stress in general as seen in Selye's cases (1).

To test this question animals were adapted to a different type of stress, namely, the severe effects of intraperitoneal glucose injections. The following procedure, shown by Reiss *et al.* (8) to produce resistance to the shock-inducing effects of intraperitoneal glucose solutions, was used: on four alternate days 1.5 cc. of 40 per cent glucose solution per 100 grams body weight were injected intraperitoneally. On the second day after the last glucose injection water intoxication tests were made.

There was again a consistent and slight superiority of the glucose-adapted animals when compared with normal controls. The differences in water excretion were not significant and those in body temperature failed slightly to reach acceptable criteria of significance, i.e., the P value of Fisher exceeded 0.05. The existence of a true difference would be consistent with the interpretation of related experiments by Karady *et al.* (15). The important point is, however, that the glucose-treated animals did not get along nearly as well as those adapted to water; and it can be concluded that while the acquired resistance to water administration may be in small part a resistance to stress in general, it is largely a resistance to water (plus stomach tubing) in particular.

Discussion. It might be considered that a normal animal, hydrated to the limit of tolerance, would excrete water at the maximal possible rate if its survival depended on it. There are, however, numerous factors which can increase this "maximal" diuretic rate of normal animals. Various hormones—adrenal cortical (9, 4), thyroxin (5) and epinephrine (6)—can effect such an increase above normal levels. In addition it is shown here that an adaptation to water administration, to the passage of a stomach tube and perhaps to glucose injections have the same general result. It might be thought that these latter influences act in a non-specific manner by increasing resistance to stress in general, since various other non-specific stresses have been reported to influence in similar fashion the diuresis induced by intravenous saline (15). This idea does not seem to be borne out, however, when the different order of effectiveness of the different influences studied here is considered. Thus, while glucose-adaptation was not clearly helpful, stomach-tube adaptation was, but the latter much less effective in turn than water (plus stomach tube) adaptation. Judging by superficial appearances one would think that the glucose injections would provide the most intense and

drastic of these stimuli. Something more seemed to be involved than a non-specific "alarm reaction."

The increased rate of diuresis seen in animals adapted to water is not the only cause, and perhaps is only the consequence of the increased resistance to water intoxication. Evidence for this is seen in these and in previous experiments. When water intoxication is established in severe form (low body temperature, etc.) diuresis subsides as we and others (16) have seen; if the toxic symptoms do not appear in severe form rapid diuresis continues. Some diuretic agents —e.g., epinephrine (6)—are weak in preventing water intoxication. Adrenal cortical hormones, on the other hand, have slight if any effect on the uncomplicated diuresis that follows the administration of small doses of water; but when large doses of water are forced they can prevent water intoxication and this is associated with but not necessarily caused by an increased diuresis. In animals adrenalectomized for a week doses of cortical hormones sufficient to palliate water intoxication have slight if any effect on the very sluggish water excretion (Gaunt, 1944; Hays and Mathieson, 1945). The fact that the augmented diuresis seen in water-adapted animals is much more apparent after large than small amounts of water is also perhaps largely due to a reduction in the "toxicity" of water which permits diuresis to continue. Hence, as also seen in the experiments with vasopressin, there are factors in the resistance to excess water independent of the rate of diuresis.

The adaptation provided by mere passage of a stomach tube is of some special interest. The insertion and withdrawal of a stomach tube, in experienced hands, can be done in a few seconds and appears to be an innocuous act. It is, however, probably somewhat painful and in animals unaccustomed to it produces excitement. An attractive but possibly misleading explanation is suggested by the finding of Theobold (10), elaborated by Theobold and Verney (11) and more recently by Haterius (12) and Hare (13) that afferent stimuli can be antidiuretic influences. (The reflex mechanism involved possibly works through the neurohypophysis (11, 12) although Hare suspects other means.) The passage of a stomach tube might be expected to serve as such an antidiuretic sensory excitant, and if so the adaptation to a stomach tube could be due to the abolition, through loss of fear and reduction of excitement, of these antidiuretic reflexes.

Whether such influences are really of importance or not, depends upon whether the resistance to water in these cases was actually due to an increased diuresis. Since the increase in water excretion in the animals adapted to a stomach tube was of questionable significance, as well as for reasons discussed above, the rôle of diuresis *per se* can be questioned.

The experiments on adrenalectomized animals show again that water diuresis is severely inhibited in the absence of adequate amounts of cortical hormones. Our results do not completely preclude the possibility that the adaptation to water reported here is largely a reflection of an increased adrenal cortical activity, but it is equally or more probable that the adaptive mechanism, whatever it may be, is simply unable to act when cortical hormones are not present. The latter interpretation receives support from the fact that one difference is apparent

between the protection afforded by water adaptation and that provided by an excess of cortical hormones: the increased rate of diuresis in adapted animals, as seen above, can be fully inhibited by pitressin, while that in animals given cortical hormones is only partially inhibited (unpublished observations).

SUMMARY

By administering water orally in increasing but non-toxic doses for five days, the resistance to water intoxication can be increased well above normal in rats.

This acquired resistance is due partly but not entirely to adaptation to the effects of passage of a stomach tube. Adaptation to damaging doses of intraperitoneal glucose solutions provided slight if any protection against water intoxication.

The resistance to water intoxication in adapted animals was not dependent entirely upon the characteristically increased rate of diuresis. When diuresis was inhibited by vasopressin adapted animals were shown to have less marked toxic symptoms at a given water load than normal ones. It was not clearly established that there was an increased rate of diuresis in adapted animals given small nontoxic doses of water.

Removal of the adrenal glands abolished largely the protective effects of adaptation to water, but the conclusion is not justified that the adaptation was mediated through the adrenals.

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BLOOD PRESSURE RESPONSE TO ACUTELY INCREASED PRESSURE UPON THE SPINAL CORD[1]

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Increasing the pressure on the portion of the spinal cord which gives rise to the thoracico-lumbar (sympathetic) outflow elicits a great rise in blood pressure, presumably brought about by asphyxial change in the spinal cord. This particular observation has not been reported, although a great deal of experimental work has been done on the effects on blood pressure of acutely increased intracranial pressure. In somewhat fewer number studies on general asphyxia in the spinal animal have been published, and within the last fifteen years some investigations on the functional effects of compression-produced asphyxia of the spinal cord below the lower thoracic region have been conducted.

We shall describe here the blood pressure response which results when the spinal cord is subjected to acutely increased pressure, and compare this response with that obtained in the same animal by raising the intracranial pressure. The comparison was made possible by the fact that under our experimental conditions increased intracranial pressure was not effectively transmitted to the vertebral canal.

METHODS. The operative and subsequent experimental procedures were performed as acute experiments on cats anesthetized with chloralosane, 55 to 65 mgm. per kgm. The trachea was cannulated at the start of the proceedings. Artificial respiration was instituted after all preparatory steps were finished and was maintained at a steady level throughout the remainder of the experiment.

Blood pressure was taken from the femoral artery and measured with a mercury manometer the inside diameter of which was 2.5 mm. A change in blood pressure of 200 mm. Hg would require the exchange of 0.5 cc. of fluid between the blood vessel and the cannula. A solution of 4 per cent sodium citrate introduced from a small syringe connected to the system was used sparingly to prevent clotting.

The pressure on the spinal cord was increased in most experiments by forcing 0.9 per cent sodium chloride solution into the spinal dural sac. For this procedure, the dura was slit open transversely at the lower level of L4 segment. A portion of spinal cord about 1 cm. long extending rostrally from the dural slit was removed from within the dura and a glass cannula tipped with a thin-walled, side-perforated piece of metal tubing was tied into the dural sac.

[1] The work described in this paper was done under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Northwestern University.

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Intracranial pressure was increased by forcing 0.9 per cent sodium chloride solution into the cranium through a brass cannula screwed tightly into the parietal bone. The tip of this cannula was perforated at the sides only, and with the cannula in position these vents opened subdurally.

Fluid pressure was generated and controlled by means of a sphygmomanometer bulb connected to the air space above the fluid in a small aspirator bottle. The tube leading from the bottle branched dichotomously at three points. The first side branch connected to a mercury manometer which was provided with a scale for direct reading of pressure. The second side branch led to the manometer which recorded on the kymograph. From the third point of division one branch was connected to the spinal intradural cannula and the other to the intracranial cannula.

Results. In an early experiment in which the vertebral column was intact and only the intracranial cannula was in place, intracranial pressure was raised and released repeatedly over a stretch of four and one-half hours. At about the middle of this period intracranial pressure was maintained at a level well above blood pressure for 15 minutes. At no time subsequently did increase of intracranial pressure cause any blood pressure elevation. At the end of the four and one-half hour period the dorsal atlanto-occipital membrane was exposed. Part of the vermis of the cerebellum and the caudal part of the medulla could be seen to be herniated through the foramen magnum. When the dorsal atlanto-occipital membrane and the dura were removed no excess fluid escaped. The parietal cortex herniated somewhat through a trephine hole when the dura was incised. The cranial half of the dorsal arch of the atlas was rongeured away and the spinal cord was severed through the cranial part of C1 segment. The brain was removed and found to be very edematous and riddled with punctate hemorrhages. Thus, it seemed that the brain had been quite effectively destroyed by asphyxiation. The brass intracranial cannula was screwed into the cranial opening of the vertebral canal, destroying the cranial one or two cervical segments of the spinal cord. When the pressure in the vertebral canal was raised through this cannula, blood pressure rose from 40 mm. Hg up to 110 mm. Upon a subsequent increase in pressure in the vertebral canal the blood pressure rose from 31 mm. Hg up to 107 mm. The experiment was concluded when the animal was inadvertently asphyxiated. There was no blood pressure rise accompanying this general asphyxia. Post-mortem laminectomy was performed at about T10. When fluid was forced through the cannula at the upper end of the vertebral canal it escaped freely from the extradural space at the site of the removed laminae, and when the dura at this site was opened the fluid escaped more readily still from the subdural space.

In all the remaining experiments the pressure on the spinal cord was applied through a cannula attached as described in the section on *Methods*. Preliminary experiments showed, as was expected, that fluctuation of spinal intradural pressure produced by introduction of fluid at the level of L3 segment appeared instantly and without diminution in a mercury manometer attached intradurally at the level of C6 or 7 segment.

Figure 1 shows clearly that the blood pressure level assumed when spinal intradural pressure was raised was very definitely a function of the relative level of the spinal intradural pressure. In this experiment, the dura was tightly ligated at T3, severing the enclosed spinal cord. Thus the dural sac into which the cannula led contained a length of spinal cord extending from the middle of T3 to the middle of L3. In that portion of the experiment during which was obtained the record of which figure 1 is a segment, the spinal intradural pressure was increased stepwise from the normal level to 174 mm. Hg over a period of $11\frac{1}{2}$ minutes. The mean blood pressure rose from 72 mm. Hg to 186 mm. Hg, always assuming a level above the spinal intradural pressure. In this cat the vagus nerves were intact. Reflex cardiac inhibition, if present, did not assert itself above other factors which resulted in an increase in heart rate.

In other experiments the brain was asphyxiated by prolonged compression and the spinal intradural pressure was then elevated without confining it to a

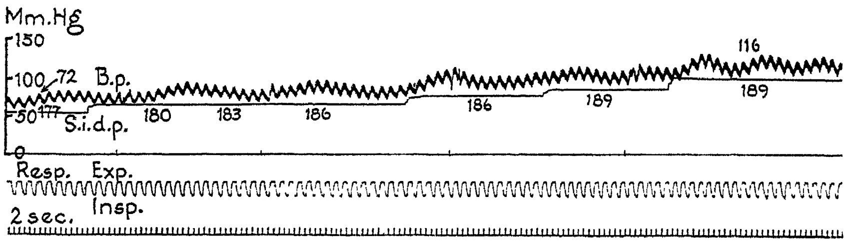

Fig. 1. Description in text above. This is the only animal which was not given artificial respiration. Respiration was recorded by means of an intrathoracic trocar connected to a tambour.

In all figures the single signal on the base line indicates the injection of 0.1 to 0.2 cc. of sodium citrate solution into the blood pressure recording system. *B.p.* is the blood pressure tracing and *S.i.d.p.* is the record of spinal intradural pressure. The numbers inscribed above the blood pressure tracing indicate the magnitude of the blood pressure at that point. The numbers below the tracing are heart rate in beats per minute for a 10 second interval in the tracing above the number.

closed spinal dural sac. Upon other occasions a ligature severing the spinal cord and closing the dural sac was placed at C6 or 7. In one cat the brain was asphyxiated and blood pressure responses to raised spinal intradural pressure were recorded. Then a ligature was tightened at C6 and blood pressure responses were again recorded. In all these experiments the vagus nerves were cut, through or just above the inferior ganglion. Spinal intradural pressure was raised within a fraction of a second to two minutes to a level exceeding the maximum blood pressure level attained, and was maintained for from one to three minutes.

Results were essentially the same in all preparations (fig. 2). The initial blood pressure levels ranged between 40 and 100 mm. Hg. Blood pressure increased rapidly when spinal intradural pressure approached the mean blood pressure. Maximum absolute blood pressure values attained ranged between 175 and 260 mm. Hg and the increments ranged between 120 and 185 mm. Hg.

The blood pressure was maintained at a plateau approximating the maximum value for from one to two and one-half minutes while the spinal intradural pressure was held above it. Then it began to decline rather rapidly. Repeated elicitation of the response and periods of asphyxiation of the spinal cord diminished the response.

If the spinal cord was still in good condition, the heart rate was augmented during the greater part of the pressor response. The maximum increment of between 26 and 84 beats per minute usually occurred after the first 40 or 50 seconds of the time that the blood pressure was at its plateau. If the blood pressure rose at the rate of at least 5 or 10 mm. Hg during the first 10 seconds after the elevation of spinal intradural pressure, the heart rate usually diminished by 2 to 6 beats per minute during the first 10 seconds. The greatest slowing occurred during the first half of this 10-second period; during the second half the rate usually began to pick up and continued to accelerate if the blood

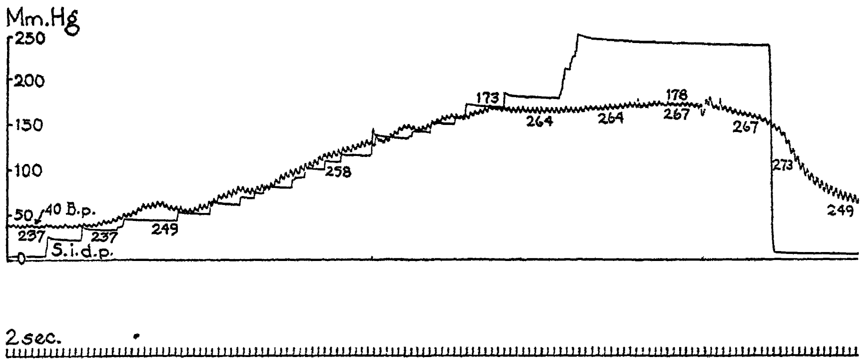

Fig. 2. The dural sac was closed and the spinal cord severed at C6 by a ligature. Two responses to increased spinal intradural pressure were elicited previous to the one recorded above.

pressure climbed steadily. The maximum heart rate, however, never occurred until after the blood pressure had leveled off at its plateau.

In these same experiments, a rapid release of spinal intradural pressure before the blood pressure began to fall resulted in a decline in blood pressure, usually of immediate incipiency, often accompanied by a cardiac acceleration of 3 to 6 beats per minute lasting for about 15 to 30 seconds.

This slight cardiac deceleration or acceleration often occurred also when the blood pressure increased or decreased abruptly during secondary fluctuations in spinal intradural pressure; when the blood pressure fell from its maximum if the pressor response had spent itself under a maintained spinal intradural pressure; and when the blood pressure rose abruptly in response to section of the cervical spinal cord or to caudally directed traction on the dura in the upper lumbar region, and then fell again.

When the spinal cord was somewhat deteriorated following repeated episodes of increased spinal intradural pressure or when the cord had been partially as-

phyxiated by a prolonged period of compression, an increase in spinal intradural pressure produced a good pressor response, albeit sometimes slightly reduced, with little or no cardiac acceleration (figs. 3 and 4).

Both adrenal glands were removed intact from an animal at a time when the central nervous system was partially deteriorated and heart action was poor. Blood pressure was 45 mm. Hg. Spinal intradural pressure was raised to 100 mm. Hg whereupon blood pressure went to 112 mm. When the spinal intradural pressure was released the blood pressure fell to its original level. This response was elicited several more times.

When pressure was raised through the intracranial cannula and the pressure in the spinal dural sac was measured through the spinal intradural cannula, it was found that the spinal intradural pressure reached a maximum of 35 mm. Hg when the intracranial pressure stood at 175 mm. Hg. No further rise of spinal intradural pressure occurred when the intracranial pressure was elevated gradually to a peak of 250 mm. Hg.

Pressor responses were compared in cats in which intracranial and spinal intradural pressures could be raised independently or simultaneously and in which both vagus nerves were sectioned and carotid sinuses and bodies denervated. In general, increased spinal intradural pressure evoked elevation of blood pressure to absolute levels as high as or only slightly lower than those accompanying increased intracranial pressure. The blood pressure increment tended to be somewhat greater in increased spinal intradural pressure than in increased intracranial pressure because of the lower initial blood pressure level in the former condition. The highest absolute blood pressure values occurred when intracranial and spinal intradural pressures were raised simultaneously, perhaps because this was the first response tested. In other aspects the pressor response to elevated intracranial pressure was the same as that already described for spinal intradural pressure except that the heart rate usually was not accelerated as greatly and the response clearly suffered more from repeated elicitation and prolonged asphyxia (fig. 4) in the case of intracranial pressure.

Discussion. It seems evident from the results that the blood pressure rise which we have described is a response to local anemia of the spinal cord induced by compression of this organ. It may be thought that the immediate stage of the pressor response to abrupt elevation of spinal intradural pressure can be accounted for by a stretching of the ventral rootlets as the dural sac is expanded. On anatomical grounds we think this is unlikely. The dura around the part of the cord with which we are dealing—T1 to L3—is normally close-fitting and taut; and the serrations of the dentate ligaments, which at every intersegment attach the dura to a lateral, longitudinal, ligamentous band of pia and arachnoid, are surprisingly strong and unyielding. Furthermore, all of the rootlets except some emanating from cord segments T10 to L3 run an oblique course between the spinal cord substance and the line of dural reflection; hence the effectiveness of any lateral displacement of the dura in stretching rootlets would be minimized. In addition, rapidly elevated intracranial pressure, which exerts no mechanical action on the spinal cord, elicits a pressor response which is char-

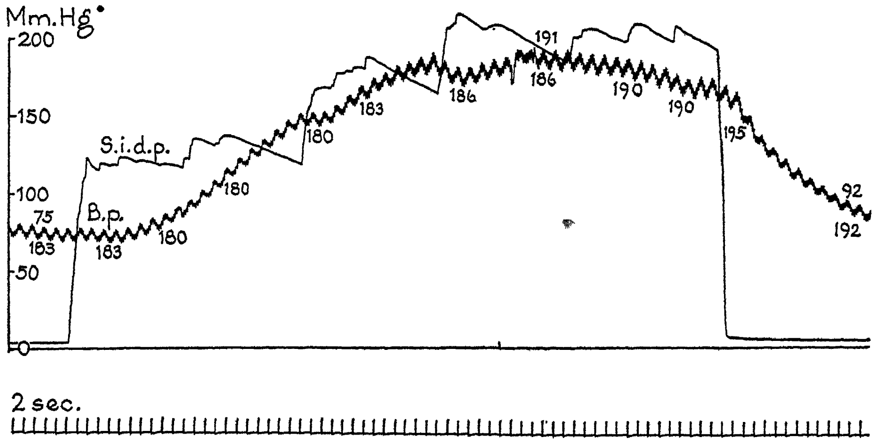

Fig. 3. The brain of this cat was asphyxiated, following which three responses to increased spinal intradural pressure were elicited. Then a ligature was tightened about the dura and cord at C6. Two more responses were elicited, following which the spinal intradural pressure was allowed to remain well above blood pressure for 10 minutes. The above response was the third after this asphyxial period.

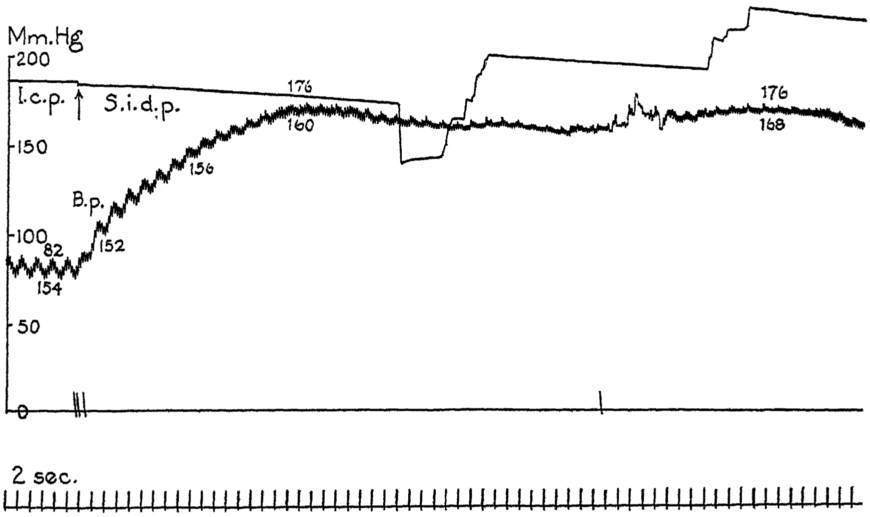

Fig. 4. Previous to the time at which this record was obtained, the pressor response to increased intracranial pressure was elicited three times. On the third occasion the intracranial pressure was maintained well above blood pressure for half an hour. Raising the intracranial pressure subsequently elicited no response. The blood pressure response to spinal intradural pressure was elicited four times after the asphyxiation of the brain. The fourth period of spinal intradural pressure elevation was prolonged for half an hour. The response shown above was the second after this and was obtained as follows: With the line to the intracranial cannula open and the line to the spinal intradural cannula clamped shut, pressure was increased. There was no response to elevated intracranial pressure (*I.c.p.*). The line to the spinal intradural cannula was then opened as the line to the intracranial cannula was clamped. This switch is indicated by the triple signal and the arrow.

acterized by the same promptness as that which accompanies spinal cord compression.

Though we cannot with certainty specify the peripheral factors which are responsible for the blood pressure rise, we believe our records indicate that the major rôle is played by a vasoconstriction of direct nervous origin. Direct nervous augmentation of the heart beat and the action of adrenalin on the heart and on the blood vessels probably contribute to the pressor response.

The slow waves sometimes imposed on the blood pressure record (fig. 1) resemble Traube-Hering waves, but whether or not they are really of central origin has not been checked. If they do represent rhythmic alterations in tone of a vasomotor center, that center in these cases resides in the spinal cord.

The slight, transient cardiac deceleration or acceleration which usually accompanied rapid changes in blood pressure (p. 581) seem to be intrinsic responses of the heart inasmuch as they coincided with the beginning of the blood pressure changes, with no appreciable latency, in animals in which the vagus nerves were cut and the cervical spinal cord was transected. The responses may be the purely mechanical, momentary consequences of the heart, which has been maintaining a certain power output per beat, suddenly being confronted with either more or less work to do.

Increased intracranial pressure, under the conditions of our experiments, is not transmitted to the vertebral canal. This is shown by direct measurement and observation and by the fact that when the brain has been completely asphyxiated subsequent elevation of intracranial pressure does not elicit a pressor response whereas increase of spinal intradural pressure elicits a large response. Naunyn and Schreiber (18), in an inadequately described experiment preliminary to their principal investigation, connected two cannulae to the subarachnoid space of the dog, one through the parietal bone and the other in the region of the cauda equina. When pressure was increased in the cranium the pressure in the spinal subarachnoid space followed it within a few seconds at a level only 2 to 5 mm. Hg lower. When pressure was increased in the spinal subarachnoid space, intracranial pressure rose to only a fraction of the spinal value. We do not think this observation has ever been quoted, and there is no indication that those who have experimentally raised intracranial pressure through a cannula over the cerebrum have been aware of it. Nevertheless, the general silence of these investigators on the subject of transmission of pressure seems to indicate that they accepted transmission as a truism. Still, compression of the spinal cord was never suspected of playing a rôle in the blood pressure rise accompanying experimental intracranial pressure elevation. Cushing (2,3,4) even increased intracranial pressure through a cannula over the cerebrum, cerebellum or upper cervical spinal cord. But whenever the cause of the increased blood pressure was assigned, it was attributed to anemia of the medulla (2, 3, 4, 6, 7, 8, 9, 10, 11, 23).

We doubt that the observation of Naunyn and Schreiber (18) is valid even for the dog. Cushing (2, 3, 4) found that severing the spinal cord in the dog at the level of the atlas prevented the blood pressure response to increased intra-

cranial pressure. Had fluid welled from the site of operation it doubtless would have caught the attention of the experimenter. Also, Cushing (2, 3, 4) temporarily paralyzed the bulbar centers by injecting cocaine into the neighborhood of the fourth ventricle through a needle inserted through the atlanto-occipital ligament. Increase of intracranial pressure did not then affect blood pressure until the cocaine effect began to wear off. In light of our findings it seems that the intracranial pressure was not transmitted to the vertebral canal in these experiments of Cushing.

Montgomery and Luckhardt (17) increased intracranial pressure by forcing fluid into the cranium through a cannula screwed into the skull overlying the cerebrum. They found that the knee jerk usually diminished or disappeared as the intracranial pressure approached blood pressure in the dog. When the spinal cord below the upper lumbar level was compressed by forcing fluid into the isolated corresponding part of the vertebral canal, the knee jerk in a majority of instances was enhanced, often enormously exaggerated, for about 30 seconds, then was abolished. On the one hand it seems that their principal belief was that the lumbar portion of the spinal cord was deprived of blood when the intracranial pressure was raised and that the resulting asphyxia of the knee jerk center was the cause of the alterations in the reflex. On the other hand they briefly postulated that the prompt diminution of the knee jerk upon marked intracranial pressure increase might be due to asphyxial stimulation of inhibitory neurons arising in the brain and acting upon the knee jerk center. It seems quite likely, however, that all of the influence of increased intracranial pressure on the knee jerk was due to the effect of compression anemia on the inhibitory and augmentative areas in the bulbar reticular formation which Magoun (15) has recently described.

Dixon and Halliburton (6) and Freeman and Jeffers (9) increased intracranial pressure by injecting fluid into the cisterna magna. Though these investigators attributed the blood pressure rise to anemia of the medulla there is a good possibility that here the fluid was free to enter the spinal intradural sac.

Montgomery and Luckhardt (17), Porter, Blair and Bohmfalk (19) and Van Harreveld and Marmont (22) have studied the effect of compression asphyxia of the caudal part of the spinal cord on reflex activity. Montgomery and Luckhardt (17) confined the increased pressure to the segments below the upper lumbar. They reported that there were no blood pressure changes. Porter, Blair and Bohmfalk (19) worked with cats in which the brain and the spinal cord down to a lower thoracic level were destroyed. They found that increased intraspinal pressure raised the blood pressure, the increment ranging from 2 to 76 mm. Hg. Van Harreveld, in a series of papers of which the one referred to above (22) is the first, has elevated the pressure on the spinal cord below T9 or T10 in cats but has not observed blood pressure.

Reports on blood pressure alterations in spinal animals during general asphyxia seem at first to be inconsistent but the inconsistency disappears when some of the variables are considered. Luchsinger (14) found that in rabbits and cats with spinal cord and vagi cut, suspension of artificial respiration caused

a delayed slight rise of blood pressure of not over 45 mm. Hg, then a fall. If artificial respiration was reinstituted just before death the blood pressure generally rose to a higher level than before and in shorter time, and then fell slowly. In rabbits similarly prepared, Konow and Stenbeck (13) observed a maximum blood pressure rise during asphyxia of about 38 mm. Hg. With the spinal cord destroyed in addition, asphyxia caused only a steady fall in blood pressure. Sherrington (21) described in detail the pressor response to asphyxia in decapitated cats. In the first 2 to 3 minutes there was a slow, slight rise followed by a gradual fall. Then the blood rapidly darkened, the heart accelerated and the pressure reached as much as 170 mm. Hg. The heart suddenly slowed, an occurrence unchecked by atropin. If ventilation was resumed, the heart beat suddenly became rapid and the blood pressure rose transiently to nearly 300 mm. Hg. Kaya and Starling (12) showed that in spinal dogs breathing nitrogen plus less than 5 per cent oxygen there was a marked rise of arterial pressure. In case the heart was in feeble condition at the outset the blood pressure fell as soon as or shortly after vasoconstriction occurred. This vasoconstriction was demonstrated either plethysmographically or by administering oxygen or air in which event the immediately revived heart pumped blood against the already present vasoconstriction and the blood pressure rose suddenly. This observation serves as a partial basis for understanding the variability in the blood pressure response to general asphyxia in the spinal animal. Kaya and Starling (12), Mathison (16), and Cathcart and Clark (1) demonstrated that there was no blood pressure response in spinal animals administered 10 per cent or 12 per cent carbon dioxide plus adequate oxygen. Mathison (16), working with decapitated cats, found that when the inspired carbon dioxide was high enough the blood pressure rose greatly due to vasoconstriction. The vasoconstriction evoked by cessation of artificial respiration, low oxygen, or high carbon dioxide plus adequate oxygen was dependent upon the spinal vasomotor centers. Carbon dioxide from 10 per cent to 50 per cent gave usually an initial depression of the activity of the heart followed by an increase. Percentages of carbon dioxide below the threshold value for the spinal vasomotor centers sometimes caused a rise in blood pressure, less than that due to vasoconstriction, due to increased cardiac output. Carbon dioxide acting directly on the blood vessels caused dilatation.

Czubalski (5) and Seppä (20) published papers dealing with the blood pressure response to asphyxia in spinal animals but added little to the subject. Both reported the effects of asphyxia in the spinal animal after removal of the adrenals. Czubalski (5) found no rise in blood pressure; Seppä (20) observed a slow rise to 41 mm. Hg from a base of 17 mm., and a peak of 43 mm. Hg after artificial respiration was resumed. It is possible that their preparations were badly deteriorated, which would account for the poor responses.

It is plain that the blood pressure change in general asphyxia of the spinal animal depends upon several variables which becloud the response of the spinal cord. With these variables excluded it becomes evident that the spinal cord is capable of responding in a remarkable fashion to asphyxia so as to effect a great rise in general blood pressure.

SUMMARY

When spinal intradural pressure is raised acutely in animals in which the brain has been rendered functionless by compression asphyxiation or in which the spinal cord has been severed by ligature in the cervical region, a pressor response occurs which is comparable to that elicited by increased intracranial pressure with the central nervous system intact.

Since under the conditions of our experiments elevated intracranial pressure is not transmitted to the vertebral canal, the responses can be attributed to anemia of the spinal cord in the case of raised spinal intradural pressure, and to anemia of the medulla in increased intracranial pressure.

The response to increased spinal intradural pressure occurs after the adrenals have been removed. A good response is obtained with little or no cardiac acceleration if the response has been repeatedly elicited or if the spinal cord has been partially asphyxiated.

The spinal cord structures responsible for the response to increased spinal intradural pressure are more resistant to inactivation by prolonged asphyxia than are those in the medulla which respond to elevated intracranial pressure.

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AVERAGE FOOD CONSUMPTION IN THE TRAINING CAMPS OF THE UNITED STATES ARMY (1941–1943)

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The food consumption of soldiers in the training camps of this country during 1917–1918 has been adequately described by Murlin and Hildebrandt (1) and Howe, Mason and Dinsmore (2). Since that time, interest in food consumption studies has continued and expanded because of *a*, our increased knowledge of the physiologic importance of food constituents in general, and of vitamins and minerals in particular; *b*, the current scientific prominence of problems concerning human requirements for nutrients and the composition of foods in relation to those requirements, and *c*, the renewed attention given to food by the public due to rationing, supplying the Army, supplying other countries, etc.

Studies such as those carried out by Stiebeling and Phipard (3) and by McCay (4) have contributed significantly to our knowledge of food habits and food requirements. It is the purpose of this report to record the results of food consumption surveys in 455 Army messes during the period 1941–1943. It is our belief that this information may serve as a sequel to the original studies carried out during World War I, and may perhaps contribute also to our rapidly accumulating knowledge of the present-day food habits and food consumption of active young adults.

Since the middle of the year 1941 through the end of 1943, we have accumulated data on various phases of messing operations in the training camps of the army in this country. The data have been obtained by scientific specialists who had been recently commissioned as Nutrition Officers in the Medical Department of the United States Army. Generally speaking, the data have been obtained over short unannounced periods of time ranging from six to ten days in each mess. The results for each Post have been reported as a thirty-day average of these individual surveys, and are considered to provide accurate representative information on average food consumption. Fifty Posts and 455 messes have been surveyed in this manner.

The results of these nutritional surveys have been reported in terms of food groups, the bases for which are similar nutritive content, special function in the diet, or unique contribution to the diet, as described elsewhere by Howe, Pritchett and Berryman (5). Nutritional evaluation of the quantities of food consumed have been carried out by a short method of evaluation of food groups described by Berryman and Chatfield (6) and by Berryman and Howe (7). The values obtained have been checked periodically by the use of the National Research Council tables of food composition (8).

Table 1 indicates the average quantities of the various food groups consumed in the mess per man per day during the periods under survey. The second

column in table 1 indicates the distribution of waste by food groups in terms of the percentage of the total amount of food used.

The approximate nutritive value of the food consumed for the periods is shown in table 2. It will be noted that the quantities of essential nutrients as calculated are liberal. However, it must be emphasized that such calculated values cannot indicate the extent of losses of the heat-labile, water-soluble nutrients due to

TABLE 1

Average quantities of food consumed in army messes as determined in 455 messes during 1941–1943

FOOD GROUPS	CONSUMED	PER CENT EDIBLE WASTE*
	lbs. per man per day	
Meats, fish and poultry	0.909	7.5
Eggs	0.149	6.2
Milk and milk products (fluid milk equivalents)	1.016	1.6
Fats, butter and spreads	0.083	0.7
Fats, other	0.069	4.5
Sugar and syrups	0.262	1.0
Cereals and grain products	0.567	11.1
Beans, other legumes, dry, nuts, dry	0.055	10.5
Vegetables, leafy green or yellow	0.359	16.4
Tomatoes	0.155	3.5
Citrus fruits	0.248	1.3
Potatoes	0.561	12.9
Vegetables, other than leafy green or yellow	0.252	10.8
Fruits, other than citrus	0.358	2.0
Fruits, dried	0.026	4.7
Total edible waste-pounds per man per day		0.39

$$\frac{\text{*Average edible food wasted in mess hall \& kitchen}}{\text{Average total edible food issued to organization}} \times 100.$$

preparation, cooking, and service of food. If deduction factors for such losses are applied as follows:

Thiamine	40 per cent of value of the diet
Riboflavin	15 per cent of value of the diet
Niacin	20 per cent of value of the diet
Ascorbic acid	35 per cent of value of the diet

the nutritive values become those shown in parentheses in table 2. We have considered such deductions to be conservative; more recent information on messing practices, to be described later in this paper, would indicate that the true losses are probably higher.

The various types of organizations or units surveyed had a wide range of duties and levels of activity. They include infantry, artillery, cavalry, quartermaster, military police, ordnance, engineering, medical, air corps, chemical warfare, tank,

air, induction center and others. Because the number of each type of unit differed widely (from 1 chemical warfare unit to 77 infantry units), and because the caloric value of food consumed likewise differed over a wide range (from 3132 for five induction centers to 4135 for three demolition units), the average caloric values obtained for the various types of units (table 3) have been weighted according to the number of surveys carried out on each type. The average caloric value of the food consumed in the mess, when properly weighted in this manner, was almost 3700 calories (3694) by calculation. This agrees closely with the estimate reported by Murlin and his associates during the last war (3633). As was also observed in World War I report, the calculated caloric value of food

TABLE 2

Calculated nutritive value of average food consumed per man per day as determined in 455 messes during 1941–1943

NUTRIENTS	CONSUMED
Calories	3785
Protein, gm.	125
Fat, gm.	178
Carbohydrates, gm.	408
Calcium, mgm.	923
Phosphorus, mgm.	1885
Iron, mgm.	22
Vitamin A, I.U.	11881
Thiamine, mgm.	3.12
(Thiamine)	(2.08)*
Riboflavin, mgm.	2.71
(Riboflavin)	(2.3)*
Nicotinic acid, mgm.	28.1
(Nicotinic acid)	(22.5)*
Ascorbic acid, mgm.	111.5
(Ascorbic acid)	(74.4)*

* Following deduction for conservative probable losses due to cooking (see p. 589).

consumed in the ordinary mess rarely lies outside the range of 3000–4000 calories (table 3).

The latter statement, however, deserves further explanation. For reasons adequately listed by Murlin and Hildebrandt (1), it is almost impossible to obtain an accurate record of the items consumed by the soldier outside the mess hall. Even though canteen (post exchange) purchases may be determined quite closely, they are difficult to interpret adequately because of errors arising from the fact that no one canteen is necessarily limited to the patronage of soldiers living near it, and furthermore, the purchases of one individual may be consumed by several. Over and above this additional food bought at the canteen, however, there are other sources of food supplements which are even more difficult to fol-

low, as for example the personal packages sent to the soldier by relatives or friends, and the food or refreshment bought in the nearby town.

It was estimated in 1919 that the caloric value of food bought at canteens averaged approximately 365 calories per man per day. McCay (4) reporting on sales by ships' canteens, estimates the average caloric value of food per man bought there to be approximately 400 calories. It would therefore appear justified to state that over long periods of time, the average caloric value of food consumed per day by the average soldier is probably in the range of 4000–4100 calories, i.e., 3700 + 400. However, appropriate reservation is required even here. It is obvious that certain individuals may eat considerably less or more than the averages calculated and listed above, both in the mess hall and outside.

TABLE 3

Caloric value of food consumed by different types of organizations surveyed during 1941–1943

TYPE OF UNIT	NUMBER OF MESSES SURVEYED	AVERAGE CALORIC VALUE OF FOOD CONSUMED IN THE MESS
Infantry	77	3733
Artillery	68	3295
Cavalry	7	3878
Coast Artillery (A.A.)	45	3711
Quartermaster	61	3908
Medical	24	3623
Headquarters	16	3801
Military Police	11	3719
Ordnance	5	3836
Engineers	18	3845
Transportation	37	3655
Tanks or Mechanized	30	3880
Air Corps	27	3779
Signal	18	3766
Induction Center	5	3132
Miscellaneous	3	3298
D.E.M.L.	3	4135

We have examined the data in terms of seasons. The results are again similar to those reported in 1919. The caloric intake was highest during the Fall (Sept.–Nov., 3960 calories) dropping in the Spring (March–May, 3570 calories) and rising slightly during the Summer (June–August, 3790 calories). The observation by Murlin (1) that seasonal variation caused no more than a 400 calorie deviation (in food consumed in the mess only) is corroborated by our data.

Our data on waste of edible food indicate that an average of 0.39 pounds per man per day have been discarded during the various survey periods. This figure includes water rich foods such as cooked rice, cooked beans, soups and the like. It is interesting to note that in 1919 when food conservation measures were used similar to those put into effect so successfully today, the average edible waste was reported as 0.38 pound per man per day.

During the first week of June, 1943, *simultaneous* surveys were carried out in 99 messes at 38 ground force stations, representing approximately 130,000 rations consumed. Table 4 indicates the results of these surveys, and shows similarity to those expected for the season of the year. Average caloric intakes for the various stations ranging from 2800 to 4600 calories per man per day, with a mean of 3470. The lower quantity of edible waste (0.32 lb. per man per day)

TABLE 4

Quantities of food planned and consumed in 99 army messes by simultaneous survey, June 1–7, 1943

FOOD GROUPS	PLANNED	CONSUMED	PER CENT EDIBLE WASTE*
	lbs. per man per day	*lbs. per man per day*	
Meats, fish and poultry	0.905	0.810	6.4
(Pork)	(0.113)	(0.201)	(8.2)
Eggs	0.147	0.152	5.8
Milk and milk products	1.053	1.097	1.5
Fats, butter and spreads	0.065	0.068	0.5
Fats, others	0.052	0.051	2.3
Sugar and syrups	0.241	0.241	1.2
Cereals and grain products	0.621	0.518	9.1
(Bread)	(0.361)	(0.317)	(10.5)
Beans, other legumes, dry, nuts, dry	0.055	0.038	12.5
Vegetables, leafy green or yellow	0.501	0.343	13.9
Tomatoes	0.123	0.139	3.6
Citrus fruits	0.329	0.338	0.6
Potatoes	0.650	0.533	9.7
Vegetables, other than leafy green or yellow	0.289	0.229	12.5
Fruits, other than citrus	0.292	0.259	2.7
Total	5.360	4.848	
Number of calories	3,764	3,468	
Edible waste (lbs. per man per day)			
Total			0.32
Table waste			0.16
Kitchen waste (by difference)			0.16

$$* \frac{\text{Average edible food wasted in mess hall \& kitchen}}{\text{Average total edible food issued to organization}} \times 100.$$

undoubtedly reflects the success of the Army's vigorous efforts to keep waste to a minimum.

At the time of these simultaneous surveys, data were also obtained on messing practices, i.e., total elapsed preparation times. The general impression obtained was referred to earlier in connection with arbitrary deductions applied to calculated nutritive values, namely that the length of time used in the average Army mess for preparation, cooking, and service of food frequently may be excessive.

It is obvious that proper preparation and cooking practices are of great importance in the attainment of adequate nutrition. This is particularly true in the Army, where the effect is two-fold: *a*, on nutritive content, and *b*, on palatability, and therefore on acceptance and waste of food. Table 5 indicates that those food groups normally designed to supply the daily intake of heat-labile water-soluble constituents (such as ascorbic acid and thiamine) frequently underwent excessive preparation, cooking and standing. It is apparent that such practice may easily neutralize part, if not all, of the excellent care given to the proper planning and procurement of food such as that in Field Ration A, the rough equivalent of a liberal civilian diet. Continued education and supervision of mess personnel are essential in the interests of maintaining an adequate satisfactory diet.

It would be desirable to be able to report the overall nutritional status of soldiers subsisting on the type of diet and level of nutrients characterized by Field Ration A. Unfortunately, such data obtained on a large sample of the Army are not available, except negatively in that we have never received any verifiable reports of gross nutritional deficiency symptoms within this country. In a survey conducted on a small group of active soldiers who had subsisted on Field Ration A while completing thirteen weeks of basic military training, the following biochemical findings were reported by Pollack and Berryman (9):

TABLE 5

Observations on length of time taken in preparing or cooking food and on standing time

TYPE OF FOOD	PREPARATION OR COOKING TIME			STANDING TIME
	Nominal allowance in hours	Percentage requiring		Per cent standing more than half hour
		One to two hrs.	Two or more hrs.	
Meats	2		40	62
Vegetables, leafy green or yellow	1	43	26	76
Salads	1	17	75	100
Potatoes	1	61	15	27
Vegetables, other	1	15	33	61

Average B-vitamin load test response: (4 hr. excretion of thiamine and riboflavin, immediately following intramuscular administration of sterile mixture of 1 mgm. thiamine hydrochloride, 1 mgm. of Na salt of riboflavin, and 20 mgm. nicotinamide).

Thiamine	220 mcg. per 4 hr.
Riboflavin	290 mcg. per 4 hr.
F2 Factor	Not determined

Average ascorbic acid load test response: (6 hr. excretion of ascorbic acid immediately following intravenous administration of 200 mgm. ascorbic acid).

Ascorbic acid (reduced form)	22.8 mgm. per 6 hrs.

Average B-vitamin basal urinary excretion: (4 hr. excretion of thiamine, riboflavin, and F-2 factor determined 8 hr. following previous meal).

Thiamine	33 mcg. per 4 hr.
Riboflavin	87 mcg. per 4 hr.
F2 Factor	4.0 thiamine units

Average ascorbic acid basal urinary excretion: (6 hr. excretion of ascorbic acid determined six hours following previous meal).

Ascorbic acid (reduced form)	1.6 mgm. per 6 hrs.

SUMMARY

Four hundred and fifty-five nutritional surveys have been carried out at 50 military posts of the United States Army where Field Ration A, the rough equivalent of a liberal civilian diet in which there is a wide range of all types of food, is provided. The food consumption levels reported indicate that the American soldier in the training camps of this country receives a nutritionally adequate diet, although messing operations may be of such nature as to deduct considerably from the optimum which could be attained. In the mess, the average caloric intake of the average soldier per day, as determined by evaluation of food consumed, is approximately 3700 calories. Average purchases in the canteen probably approximate 350 to 400 calories per man per day. Seasonal variations in the consumption of food probably do not exceed 400 calories (in the mess hall). Some biochemical results obtained during a survey of a small group of soldiers (50) are presented as possibly being indicative of the nutritional state of soldiers following 13 weeks subsistence on Field Ration A while carrying out the activities involved in basic military training.

Acknowledgments. The food consumption values listed represent a summary of the data reported by and vouched for by Nutrition Officers carrying out nutritional surveys at various posts, camps and stations within this country, and acknowledgment is made of their careful and diligent efforts which have made this report possible.

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BLEEDING VOLUME IN EXPERIMENTAL SHOCK PRODUCED BY PROLONGED EPINEPHRINE ADMINISTRATION, INTRAPERITONEAL INJECTION OF GLUCOSE, AND INTESTINAL STRANGULATION

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In most experimental studies on shock, survival rates or average survival periods are used for gauging the severity of the inflicted circulatory damage. Such studies require constant attendance and elaborate regulation of environmental factors throughout the survival period. They throw no light on the mechanism of circulatory failure. Studies on shock would obviously be facilitated by the measurement of some value in the surviving animals which could be taken as an index to the over-all impairment of the circulation. The possibility that the bleeding volume might serve as such an index is suggested by recent studies on hemorrhage and blood substitution (Lawson and Rehm, 1945b, c). In a complete study of bleeding volume, data are obtained not only on the magnitude, but also on the mechanism of circulatory impairment.

As used in these studies, the term bleeding volume means the fatal hemorrhage volume as determined by controlled arterial bleeding. It is equal to the prehemorrhage blood volume plus the volume recruited during bleeding, minus the liminal circulating volume, the last being the blood volume required for a minimal cardiac output. In the studies reported here, each of these volumes was measured. Control values were obtained without sacrificing the animals, by bleeding just short of circulatory standstill, and immediately re-injecting all the drawn blood. Final values were obtained by complete hemorrhage about four hours later.

Procedures designed to produce shock-like states were instituted about one hour after the re-injection of blood. Although data were obtained which show that in the absence of these procedures the effects of the first bleeding are almost completely reversed within four hours, no data were obtained on the extent of their reversal at one hour. In the shocked animals, therefore, the final values may represent a summation of the effects of the shocking procedures with residual effects of the first bleeding. For the present purposes this does not seriously limit the usefulness of the data.

METHODS. The animals used in the studies were healthy, mature street dogs. They were deprived of food and water for twenty-four hours before the experiments, and were anesthetized by an intravenous injection of 250 mgm./kgm. of sodium barbital at least three hours before the first bleeding. To ensure maximum reversal of the effects of the first bleeding, they were given 15 cc./kgm. of 0.9 per cent sodium chloride solution intravenously immediately following the barbital injection (Lawson and Rehm, 1945b).

Methods have already been given in detail for the measurement of the bleeding volume, the pre-hemorrhage blood volume, the volume recruited during the bleeding, and the liminal circulating volume (Lawson and Rehm, 1945a, b). For both the control and the final observations blood was drawn from a femoral artery in unit volumes of 2 cc./kgm. at intervals of two minutes. The control bleeding was discontinued as soon as mean arterial pressure, recorded from the other femoral artery, remained for two minutes below 20 mm. Hg. The volume which can be drawn below this level without stopping the circulation has been shown to have an average value of about 5 cc./kgm., with a range of 2 to 9.4 cc./kgm. at 0.05 probability. The blood obtained during the control bleeding was heparinized as it was drawn, and re-injection was begun immediately on reaching the end-point. Freshly drawn donor blood was used to make up the volume lost in sampling, so that the volume re-injected was always equal to the volume drawn. The final bleeding was started about four hours after the re-injection, and was continued until mean arterial pressure remained permanently below 10 mm. Hg.

"Direct" determinations of plasma volume were made at the beginning of both the control and the final bleeding, as the dilution volume of the dye T-1824. "Indirect" determinations were made near the middle of each bleeding and at their termination. The circulating cell volume at each of these determinations was computed from the percentage volume of cells in the sample, and the plasma volume, using the formula $0.775 \times \frac{\text{per cent cells} \times \text{plasma vol.}}{100 - \text{per cent cells}}$, in order to correct for the systematic over-estimation which has been demonstrated for the method (Stead and Ebert, 1942; Lawson and Rehm, 1945a).

Continuous intravenous injections of epinephrine were started about one hour after the control observations were completed, and were allowed to run for approximately two hours. The final observations were made about one hour after the epinephrine injections had been discontinued. Solutions containing 0.1 mgm./cc. were used, and were injected into a femoral vein under a constant pressure of 300 mm. Hg through a resistance adjusted to give a flow of about 1 cc./min. Data are given for dosage rates between 0.0069 and 0.0096 mg./kgm./min. Smaller doses were tried in two additional animals, without striking effects on bleeding volume, and one animal receiving a considerably larger dose died before the final observations could be made.

Intraperitoneal injections of isotonic (5.5 per cent) glucose solution were given about one hour after the completion of the first observations, in a dose of 100 cc./kgm. The fluid was allowed to remain in the peritoneal cavity throughout the remainder of the experiments.

In the experiments on intestinal strangulation, the belly was opened by a short midline incision and the wound closed with clamps before the first bleeding. About one hour after the first observations were completed, the small intestine was delivered through the wound, and its mesentery incised for the placement of strangulating ligatures, which were left in place until the final observations were finished. In about half the experiments rubber ties were applied as tightly

as possible without obliterating the arterial pulse, leaving the collateral circulation at the proximal and distal ends of the intestine undisturbed. In the remainder of the experiments, tight ligatures were applied proximally and distally to the lower one-third of the small intestine, and all the veins draining the intervening loop were dissected up and ligatured tightly. Application of the latter type of strangulation to the entire small intestine usually produced death before the final observations could be made.

Results. *Control experiments on unshocked dogs.* On the strength of data which have already been reported for partial re-injections, practically complete reversal of the effects of the first bleeding may be expected to follow re-injection of all the drawn blood, as in the present studies. If reversal is complete, the following equations should be found:

$H_1 = H_2$, where H_1 is the bleeding volume obtained on the control observations, and H_2 is the final bleeding volume. H_1 is estimated by adding 5 cc./kg. to the volume drawn in lowering pressure to 20 mm.

$Q_1 = Q_2$, where Q_1 is the pre-hemorrhage blood volume measured at the start of the control bleeding, and Q_2 is the corresponding volume at the start of the final bleeding.

$L_1 = L_2$, where L_1 is the liminal circulating volume at the time of the control bleeding, and L_2 the liminal circulating volume on the final bleeding. L_1 is estimated by subtracting 5 cc./kgm. from the volume remaining in the circulation at 20 mm. Hg, i.e., at the end of the first bleeding.

$I_1 = I_2$, where I_1 is the volume recruited to the circulation during the first, and I_2 the volume recruited during the final bleeding. Values for I are calculated by subtracting the pre-hemorrhage volume from the sum of the volume drawn plus the volume remaining at the end of the bleeding. I_1 is probably somewhat under-estimated, since the first hemorrhage is terminated before death.

Table 1 gives the data for these equations, expressed as the difference between the control and the final values. From these data equations for the change in bleeding volume may be constructed in the form: $\Delta H = \Delta Q + \Delta I - \Delta L$. It is apparent from the table that only small changes in bleeding volume occur in unshocked dogs, and that these usually result from a combination of changes in the limiting factors, rather than from a single change. ΔH must lie outside the range -3.0 to $+4.4$ cc./kgm., in order to be ascribed with assurance to anything more than the error in estimating H_1 from incomplete hemorrhage. Attention is called to the frequency with which changes of approximately equal magnitude and in the same direction occurred in Q and L. This is an extension of observations already reported on the apparent relationship between these two volumes. The liminal circulating volume appears from these data to be often considerably lower on the final bleeding. Similar observations were made in the earlier studies where incomplete re-injections were given, although the average decrease in the liminal volume was not so great. The average loss of bleeding volume in the present studies is correspondingly smaller.

Separate values are also given in table 1 for the pre-hemorrhage volumes of plasma and cells, and for the volumes of each which appear to be recruited during

the bleedings. There was no consistent difference between the initial circulating cell volume and the cell volume measured at the start of the final observations. The cells which were recruited during the first bleeding thus appear to be withdrawn from the circulation following complete re-injection. In the previous studies on partial re-injection, a large part of the recruited cells appeared to remain in the circulation (Lawson and Rehm, 1945b). The data on cell and plasma recruitment show that the volume of both was somewhat reduced on the final bleeding in the majority of instances. The total recruitment of both cells and plasma on the final bleeding averages about 75 per cent of the recruitment on the first. The average calculated hematocrit for the blood recruited on the first bleeding is 43.6, and on the final bleeding 43.1. Supplementary data on recruitment in both shocked and unshocked dogs are given in the form of the rates observed, in table 4.

TABLE 1

Control experiments in unshocked dogs

Δ values give the change in each volume during the period of observation, as the volume measured on the final bleeding minus the volume measured on the first. ΔH is the change in bleeding volume, ΔQ the change in prehemorrhage blood volume, ΔI the change in volume recruitment during hemorrhage, and ΔL the change in the liminal circulating volume. The equation for bleeding volume change is: $\Delta H = \Delta Q + \Delta I - \Delta L$. The remaining values in the table are the absolute volumes of cells and plasma circulating at the start of the first (Q_1) and the final bleeding (Q_2); and the absolute volumes of each recruited to the circulation during the first (I_1) and the final bleeding (I_2). All volumes are given as ccm./kgm. body weight.

DOG NO.	ΔH	ΔQ	ΔI	ΔL	PLASMA				CELLS			
					Q_1	Q_2	I_1	I_2	Q_1	Q_2	I_1	I_2
1	5.0	−12.6	4.3	−13.3	53.4	50.4	3.9	3.2	41.6	32.0	0.4	5.4
2	−5.1	2.7	−2.7	5.1	38.1	40.9	5.1	4.1	28.9	28.8	1.9	0.2
3	−9.0	−9.9	1.4	0.5	47.3	39.6	2.9	2.7	29.5	27.3	2.2	3.8
4	−3.0	−0.1	−4.8	−1.9	55.4	55.4	8.8	8.0	28.3	28.2	7.1	3.1
5	−1.0	−6.1	−0.5	−5.6	48.3	43.1	4.0	4.6	24.4	23.5	5.3	4.2
6	2.9	−12.0	−2.4	−17.3	60.0	50.2	4.1	3.6	35.7	33.5	2.8	0.9
7	−5.0	3.1	0.4	8.5	48.3	48.1	3.3	5.1	27.6	30.9	6.2	4.8
8	5.0	0.3	−2.4	−7.1	60.6	61.9	5.7	3.8	28.2	27.2	4.3	3.8
9	5.2	−14.6	−1.4	−21.2	67.8	56.6	7.5	3.4	33.3	29.9	2.5	5.2
10	−5.0	−15.3	−1.8	−12.1	66.5	55.4	12.0	9.8	28.9	24.7	3.5	3.9
11	7.2	0.6	−7.5	−14.1	59.8	59.8	10.1	4.9	32.2	32.8	5.0	2.7
12	−9.1	5.7	−4.0	10.8	43.6	42.7	2.7	3.4	25.7	32.3	7.9	3.2
13	3.0	7.9	−7.2	−2.3	45.8	46.1	4.8	2.8	33.5	41.1	8.9	3.7
Ave.......	−0.69	−3.87	−2.20	−5.38	53.45	50.02	5.76	4.57	30.60	30.17	4.46	3.45
S.D.......	5.55	8.17	3.21	9.96								
S.E.......	1.537	2.27	0.89	2.76								

Epinephrine shock. The essential data on epinephrine shock are summarized

in table 2, in the same form. All the animals showed large losses of bleeding volume, beyond the limits of variation found in the control experiments. The liminal circulating volume was consistently and significantly elevated. The loss of ability to recruit blood during hemorrhage was significantly greater than the loss in the control experiments. The total pre-hemorrhage blood volume does not appear from the table to be consistently affected. The changes observed in this volume are not unlike those found in the control experiments. The average equation for bleeding volume loss shows the loss to be due in about equal parts to elevation of the liminal circulating volume and to decrease in volume recruitment.

Examination of the separate values for plasma and cells reveals that during the interval between observations every animal lost plasma volume and gained cell volume. Data on the mean corpuscular volume were obtained in five experiments, and show no significant change during the interval. Expressed as percentages of the control values, the mean corpuscular volumes at the start of the final bleeding were 91, 93, 99, 109 and 110, respectively.

Plasma recruitment on the final bleeding was always greatly reduced, and in only one experiment was a positive value obtained for cell recruitment. No significance is attached to the small negative values for cell recruitment. Although it is possible that they mean withdrawal of cells from the circulation by trapping or unobserved hemorrhage during the final bleeding, it seems more likely that they represent errors in our methods of estimating circulating cell volume. The increase found in the pre-hemorrhage cell volume on the final observations was in every case of the same magnitude as the volume of cells recruited on the first bleeding. Taken in conjunction with the absence of cell recruitment on the final bleeding, the data suggest premature emptying of cell reservoirs into the active circulation, before the start of the final bleeding. Data on the rate of plasma and cell recruitment in epinephrine shock are given in table 4, and show that the rate as well as the total volume of recruitment is less than in unshocked dogs.

Shock from intraperitoneal glucose. The data are given in table 2. The losses of bleeding volume were significantly greater than in the control experiments, and of the same magnitude as in epinephrine shock. In the average equation for loss of bleeding volume each of the three limiting factors contributes an almost equal part. The liminal circulating volume was elevated, and there was a decrease in both the pre-hemorrhage volume and the volume recruited during the bleeding. Changes in the liminal volume and in the volume recruited during hemorrhage are significantly different from those observed in the control experiments.

The decrease in circulating plasma volume found at the start of the final observations was considerably greater than in epinephrine shock. The average increase in circulating cell volume was about the same. Data on mean corpuscular volume were obtained in two experiments. In one of these, with the circulating cell volume at the final observation equal to 133 per cent of its control value, the mean corpuscular volume was 142 per cent of its control. In

the other, the percentages were 124 and 129, respectively. The entire increase in the pre-hemorrhage circulating cell volume can thus be attributed to swelling of the cells. Similar treatment of the data for liminal circulating cell volume (not given in the table) shows that in these two experiments the final values were 182 and 313 per cent, respectively, of their controls. The elevated liminal cell

TABLE 2

Epinephrine shock and shock from intraperitoneal glucose

The column headings have the same meaning as in table 1. The control values were obtained before, and the final values approximately three hours after institution of the shocking procedures, the total time interval between values being the same as in table 1. The data on epinephrine shock are given in order of decreasing dosage, from 0.0096 to 0.0069 mgm./kgm./min.

DOG NO.	ΔH	ΔQ	ΔI	ΔL	PLASMA				CELLS			
					Q_1	Q_2	I_1	I_2	Q_1	Q_2	I_1	I_2
					Epinephrine							
14	−27.2	18.9	−33.0	13.1	48.4	48.0	20.6	0.5	31.4	50.7	12.6	−0.3
15	−29.1	−6.3	−7.9	14.9	46.0	35.9	4.9	1.3	23.9	27.7	3.5	−0.8
16	−28.9	0.3	−11.1	18.1	51.0	42.8	8.5	3.7	25.7	34.2	5.2	−1.1
17	−29.0	5.6	−23.0	11.6	48.7	44.2	8.6	−0.3	34.6	44.7	11.7	−2.4
18	−33.2	−2.3	−20.0	10.9	42.7	37.4	11.9	−0.1	34.0	37.0	7.3	−0.7
19	−31.1	−7.8	−5.3	18.0	55.6	46.7	10.0	4.4	41.6	42.7	3.1	3.4
Ave.....	−29.75	1.40	−16.72	14.43	48.73	42.50	10.75	1.58	31.87	39.50	7.23	−0.32
S.D.....	2.09	9.83	10.54	3.83								
S.E.....	0.85	4.01	4.31	1.57								
					Glucose							
20	−37.1	−16.0	−6.0	15.1	50.8	28.1	4.0	0.9	38.5	45.2	4.3	1.4
21	−33.2	−11.2	−11.9	10.1	53.5	31.7	6.9	0.5	30.7	41.3	5.8	0.3
22	−37.1	−14.7	−12.9	9.5	54.2	32.8	8.9	0.9	24.4	31.1	4.7	−0.2
23	−25.2	0.5	−10.3	15.4	47.6	35.1	5.3	2.4	33.6	46.6	5.7	−1.7
24	−27.3	−8.6	−14.5	4.2	43.5	25.7	7.2	0.3	28.2	37.4	9.3	1.7
25	−19.2	−11.3	−1.2	6.7	39.5	21.7	4.2	1.3	26.6	33.1	0.4	2.1
Ave.....	−29.87	−10.22	−9.47	10.18	48.18	29.18	6.08	1.05	30.33	39.12	5.03	0.60
S.D.....	7.18	5.64	4.98	4.46								
S.E.....	2.93	2.30	2.03	1.82								

volume on the final bleeding thus means that a larger number of cells are required to maintain the circulation in this type of shock.

In contrast with the findings in epinephrine shock, the magnitude of the increase in the pre-hemorrhage cell volume does not appear from the table to be related to the magnitude of the cell volume recruitment during the first bleeding, or to be associated with a complete loss of cell recruitment on the final bleeding. Data on the rate of recruitment in glucose shock are given in table 4.

Intestinal strangulation. The data are given in table 3. The loss of bleeding volume following compression of the pedicle for the entire small intestine (first group in the table) was less than the loss produced by complete venous occlusion

TABLE 3

Intestinal strangulation

The column headings have the same meaning as in table 1. The control values were obtained after the belly wall had been incised and the wound closed. The final values were obtained about three hours after the wound had been re-opened and the intestine strangulated. The first type of strangulation consists of compression, with rubber bands, of the mesenteric pedicle for the entire small gut. The second type consists of complete venous occlusion in the lower one-third of the small gut. The total time interval between values is the same as in table 1.

DOG NO.	ΔH	ΔQ	ΔI	ΔL	PLAMSA				CELLS			
					Q_1	Q_2	I_1	I_2	Q_1	Q_2	I_1	I_2
First type												
26	−17.3	−10.9	−7.2	−0.8	60.5	47.2	5.1	1.9	27.0	29.4	2.2	−1.8
27	−32.8	−15.0	−13.9	3.9	40.8	24.8	5.0	1.7	35.0	36.0	11.6	1.0
28	−17.1	−6.8	−1.6	8.7	52.7	46.7	7.2	4.7	36.8	36.0	−1.3	−0.4
29	−16.9	−13.4	−8.4	−4.9	57.7	43.7	5.7	1.5	45.2	45.8	2.7	−1.5
30	−14.9	−3.1	−4.7	7.1	47.3	42.0	4.9	3.0	24.5	26.7	5.5	2.7
31	−11.0	0.8	−7.6	4.2	41.8	40.2	5.8	1.9	28.2	30.6	2.8	−0.9
Ave.....	−18.33	−8.07	−7.23	3.03	50.13	40.77	5.62	2.45	32.78	34.08	3.92	−0.15
Second type												
32	−36.8	−12.6	−17.5	6.7	46.1	36.8	9.1	0.0	24.8	21.5	8.4	0.0
33	−52.8	−32.7	−20.0	0.1	52.2	31.3	7.8	−0.2	32.9	21.1	11.8	−0.2
34	−36.8	−12.6	−13.5	10.7	48.8	38.1	3.3	−0.4	24.0	22.1	9.4	−0.4
35	−33.0	−17.6	−14.0	1.4	44.4	30.3	4.9	−0.3	36.5	33.0	4.1	−4.7
36	−26.7	−22.4	−9.3	−5.0	49.9	36.4	4.6	−1.5	27.1	18.2	3.7	0.5
37	−31.0	−16.6	−10.1	4.3	47.6	35.7	6.6	3.3	27.0	22.3	8.0	1.2
38	−26.8	−16.0	−15.8	−5.0	52.2	41.8	9.9	−0.3	23.5	17.9	5.6	0.0
39	−45.0	−34.0	−13.4	−2.4	48.4	31.2	5.5	−0.4	38.6	21.8	7.5	0.0
Ave.....	−36.11	−20.56	−14.20	1.35	48.70	35 20	6.46	0.02	29.30	22.24	7.31	−0.45
Grand Ave...	−28.49	−15.21	−11.21	2.07	49.31	37.58	6.10	1.06	30.79	27.31	5.86	−0.31
S.D.....	12.20	9.72	5.11	5.25								
S.E.....	3.26	2.59	1.37	1.40								

of a shorter loop (second group). Neither procedure caused a marked elevation of the liminal circulating volume. The changes in this value are of the same order of magnitude as those previously reported for hemorrhage (incomplete re-injection). The average equations for bleeding volume loss show that with

both types of strangulation the loss is due largely to a decrease in the pre-hemorrhage blood volume and a lack of recruitment on the final bleeding. These two factors contribute almost equally to the observed loss of bleeding volume. Since the mechanism of the bleeding volume loss appears to be the same for the two types of strangulation, it has seemed justifiable to obtain an average equation for the entire group, in which the loss of bleeding volume is similar in magnitude to that produced by the other shocking procedures. In this equation, the elevation of the liminal volume is significantly less than that observed with either of the other types of shock, but significantly greater than that observed in the control experiments. The loss of pre-hemorrhage volume is significantly greater than in the control experiments or in epinephrine shock, but not significantly different from that observed in glucose shock.

In all cases circulating plasma volume at the start of the final bleeding was found to be decreased. Circulating cell volume was usually slightly increased

TABLE 4

The rate of recruitment of plasma and cells during the control and the final bleeding

The values given are average rates. The grand averages for the three groups of shocked animals, and the averages for the unshocked group, are given with their standard errors.

CONDITION	NO. DOGS	PLASMA RECRUITMENT		CELL RECRUITMENT	
		1st hem.	Final hem.	1st hem.	Final hem.
		cc./kgm./min.	*cc./kgm./min.*	*cc./kgm./min.*	*cc./kgm./min.*
Unshocked........	13	0.124 ±*0.015*	0.091 ±*0.010*	0.096 ±*0.014*	0.068 ±*0.009*
Epinephrine.......	6	0.232	0.061	0.157	−0.027
Glucose....	6	0.123	0.040	0.100	0.027
Strangulation	14	0.140	0.029	0.125	−0.010
Shocked (recapitulated)..........	26	0.158 ±*0.013*	0.039 ±*0.010*	0.127 ±*0.011*	−0.005 ±*0.009*

by the first type of strangulation, but was always decreased by the second. Postmortem examination revealed gross extravasations of blood within the mesentery, and large amounts of bloody fluid in the lumen of the completely occluded loops. In the other type of strangulation, the mesentery was edematous but usually extravasations larger than petechiae were not found, and the amount of bloody fluid within the lumen appeared to be less.

Both the rate (see table 4) and the absolute volume of plasma recruitment were reduced on the final bleeding. In over half the experiments, final values in the neighborhood of 0 were found for cell recruitment. The loss of ability to recruit cells during hemorrhage appears from the table to be equally complete with the two types of strangulation.

The relationship between bleeding volume and arterial pressure in shock. In figure 1 the percentage change in bleeding volume in each of the foregoing experiments is plotted against the percentage change in mean arterial pressure.

The pressures used for this purpose were read at the beginning of the first and the final bleeding. It is apparent from the figure that there is no close correlation between the two variables, but that in general, as might be expected, the larger losses of bleeding volume are associated with large decreases in mean arterial pressure. In the control experiments both changes were small, the average loss of pressure exceeding the average loss of bleeding volume. In glucose shock and intestinal strangulation, the average loss of bleeding volume was greater than the

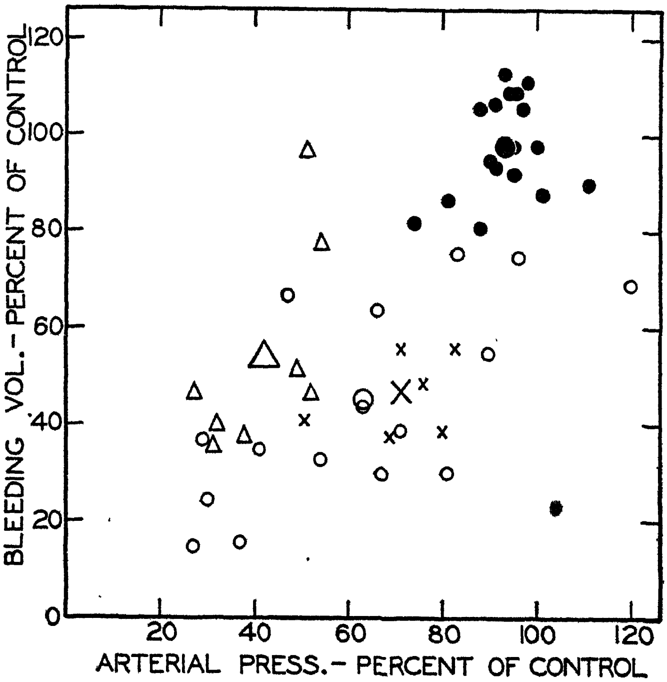

Fig. 1. Bleeding volumes are the final values, expressed as percentages of their controls Mean arterial pressures are the readings at the start of the final bleeding, given as percentages of the readings at the start of the first bleeding. Solid circles give the data for unshocked dogs, open circles for intestinal strangulation, crosses for glucose shock, and triangles for epinephrine shock. The larger symbols give the averages. The figure includes data on two animals with complete venous occlusion of the entire small intestine, duration 1½ hours; and on two animals with small doses of epinephrine, which were not given in the tables. In addition, four unshocked animals are included for which the data necessary for the tables were not obtained.

average loss of pressure. In epinephrine shock, on the other hand, marked declines in arterial pressure were observed, especially with the smaller doses, with only moderate losses of bleeding volume. If a straight line is fitted to the data for all these experimental conditions, it passes below the data for the normals and for epinephrine shock, and above the data for glucose shock to an impossible intercept at 0 arterial pressure and 7 per cent bleeding volume. It does not seem probable, from inspection of the data, that any equation could be found which

would satisfactorily express the relationship between the two variables for all experimental conditions.

The rate of disappearance of the dye T-1824 in shock. The residual dye found in the circulation at the start of the final observations is shown in table 5 as a percentage of the amount which was originally injected, approximately five hours previously, at the time of the first observations. Residual circulating dye was calculated from the concentration found in plasma just before the second dye injection, and the plasma volume as redetermined by the second dye injection (Lawson and Rehm, 1945a). The values for initially injected dye were corrected for the loss in sampling during the first observations, since these samples were replaced with undyed donor blood. The average retention at the end of the five-hour period in the experiments on unshocked dogs was about 75 per cent of the net injected dye, which is somewhat higher than would be

TABLE 5

Percentage dye retention in normal and in shocked dogs

The values given are averages. Mean percentage dye retention is given with its standard error. In calculating percentage retention of dye, the net dye injected for the first observations was obtained by subtracting the dye lost in replacing samples with undyed blood. The circulating dye remaining at the time of the final observations was calculated from the dye concentration before any additional dye was injected, and the plasma volume at that time as re-determined by the injection of additional dye. The interval was measured from the first dye injection, and was not corrected for removal of dye during the first bleeding.

CONDITION	NO. DOGS	DURATION INTERVAL	DYE RETAINED—PER CENT OF NET INJECTED	PLASMA RETAINED—PER CENT OF INITIAL PLASMA VOLUME
		hours		
Normal	13	4.87	75.5 ±2.716	93.7
Glucose shock	6	4.88	69.2 ±2.612	60.6
Epinephrine shock	6	5.07	60.9 ±3.523	87.2
Intestinal strangulation	14	4.97	52.4 ±2.279	76.3

expected from any of the formulations of dye disappearance rates in normal dogs (Gregersen and Rawson, 1943). This is probably due in part to the fact that whatever dye was withdrawn from the circulation during the first bleeding could not "disappear" during that portion of the five-hour period. No attempt was made to correct for this factor so as to obtain true disappearance rates, as the uncorrected values are valid for purposes of comparison. The average dye retention observed with each of the shock procedures was considerably less than the average retention in the control experiments. Except for the studies on glucose shock, the decrease in dye retention is statistically significant.

DISCUSSION. It is hardly conceivable that progressive failure of the circulation could be initiated by any means without causing an early change in the value of one or more of the limiting factors for bleeding volume. In view of the wide diversity of means which may be employed for producing shock-like states

(see Wiggers, 1942), it seems unlikely that the primary changes in these factors will be found to be identical in all types of shock. The limiting factors for bleeding volume measure either the volume of contents of the cardiovascular system, or the activity of the system upon its contents. Any limiting factor which is primarily affected in shock, therefore, may be expected to change toward a reduction in bleeding volume. Unless compensatory changes occur in the remaining factors, bleeding volume should accordingly be reduced. It is apparent from the present data that such compensatory changes are far from complete in the types of shock studied, since bleeding volume was reduced in all of them. Since the three limiting factors for bleeding volume cannot, on the basis of the available information, be treated as independent variables, any conclusion regarding the factors which are primarily affected in each type of shock is hazardous. The data suggest, however, that a reduction in the circulating blood volume is the primary factor in intestinal strangulation, that it is only a contributing factor in glucose shock, and that systemic changes independent of the circulating blood volume are primarily responsible for epinephrine shock.

The effects of these procedures on the circulating blood volume (pre-hemorrhage volume) are for the most part in agreement with those reported by other investigators. Comparable data have been obtained for epinephrine shock by Freeman, Freedman, and Miller (1941), and by Scholz, Schultz, Pleune, Fink, Steadman and Warren (1945); for intraperitoneal glucose by Gilman (1934), Darrow and Yannet (1935), Gaunt, Remington and Schweizer (1937), and Hopper, Elkinton and Winkler (1944); and for intestinal strangulation by Aird (1938), and Evans (1943). In our experiments circulating plasma volume was decreased in all three types of shock, but no constant relationship was apparent between the magnitude of this loss and the total circulatory impairment as measured by the decline of bleeding volume. The ratio plasma volume loss: bleeding volume loss has an average value of 0.209 in epinephrine shock, 0.636 in glucose shock, and 0.412 in intestinal strangulation. The ratio of these two losses during the period of observation in the unshocked control animals is 4.970.

Complete assurance cannot be given that true values for circulating cell volume were obtained by our methods in the shocked animals. The method, when corrected for unequal distribution of cells and plasma within the vascular system, gives reasonable values for normal dogs in various stages of exsanguination (Lawson and Rehm, 1945a). If the distribution of cell and plasma volume in shocked dogs is very different from the distribution in normal exsanguinated dogs, our correction may be in error. It seems unlikely, however, that an error of this sort could be large enough to account for our findings. Circulating cell volumes were increased in our experiments in epinephrine shock, in shock from intraperitoneal glucose, and in the milder type of intestinal strangulation. In epinephrine shock the increase appears to be due to expulsion of cells from the reservoirs with retention in the active circulation; in glucose shock it appears to be due to swelling of the cells, with no significant change in their number. The increase in circulating cells in the milder form of intestinal strangulation is taken to mean that cells have been added to the circulation from the reservoirs in

excess of the volume of cells lost locally. In the more severe form of strangulation, the local loss appears to have exceeded the volume gained from the reservoirs. Our experience with the two types of intestinal strangulation is in agreement with observations of Scott (1938) on the relative amounts of local exemia produced by compression of the entire pedicle and by complete occlusion of the veins alone.

The volume of blood recruited during the bleeding, the second limiting factor for bleeding volume, is obviously a function of the rate of recruitment and the duration of the bleeding. The data given separately on the rate of recruitment in shocked animals (table 4), show that the small volumes recruited on the final bleeding are not due simply to the fact that the bleeding is of shorter duration. Further studies are needed on the factors which govern the rate of plasma recruitment. It is apparent from the data that one or more of them undergoes deterioration in all the types of shock which were studied, and that some deterioration occurs even in the control experiments. Cell recruitment during hemorrhage is a function of the volume of cells available in the reservoirs at the start of the bleeding, and of contractile mechanisms which govern their expulsion. Both the rate and the absolute volume of cell recruitment were reduced on the final bleeding in all the shock experiments. In epinephrine shock and intestinal strangulation cell recruitment seems to have been almost completely abolished by premature emptying of the reservoirs. The lesser reduction of cell recruitment in glucose shock and on the final bleeding in the unshocked animals has no obvious explanation.

The liminal circulating volume is the final limiting factor. This is the volume of blood which must be present within the cardiovascular system for a minimum cardiac output. It may be expected to increase with impairment of contractile function either in the heart or in the peripheral blood pools. It appears, from both the present data and the data previously reported, to be quite labile under our experimental conditions. In barbitalized dogs it usually declines during the period of observation if all the blood drawn for its first measurement is re-injected, the decline being less with partial re-injections. The decline is tentatively ascribed to a decrease in the depth of anesthesia, and is taken to mean improvement of contractile function either in the heart or in the peripheral pools. Although these data suggest that under our experimental conditions the liminal volume is affected by even small changes in the pre-hemorrhage circulating volume, it is obvious that the relationship between the two volumes is neither a simple one, nor constant under all conditions. In intestinal strangulation large losses of pre-hemorrhage circulating volume occurred, with only small elevations of the liminal volume, the average ratio between these changes being 7.36. In glucose shock the ratio is approximately 1.00, while in epinephrine shock it is -0.97.

The data on dye disappearance are in agreement with those obtained on epinephrine shock by Freeman, Freedman and Miller (loc. cit.). They are at variance with the conclusions of Evans, Hoover, James and Alm (1944) based on studies of dye concentration curves in intestinal obstruction. It appears

from our data that dye may disappear from the circulation nearly twice as fast in animals with intestinal strangulation as in normals, and that the disappearance rate is somewhat increased in all the types of shock studied. It is obvious that our estimates of plasma volume in shock may be in error, since the estimates are based on an assumed disappearance rate of 10 per cent per hour (see Lawson and Rehm, 1945 a). If the data are recalculated on the basis of an assumed rate of 20 per cent per hour, the values for prehemorrhage blood volume are reduced by an average of 2.0 ccm./kgm., which is not a significant revision. Revised values for the liminal circulating volume are about 5.5 ccm./kgm. lower than those reported. The liminal volume still appears to be significantly elevated in all types of shock except intestinal strangulation. Values for blood recruitment in shock are still further reduced by recalculation of the data on this basis, the revised values being about 3.5 ccm./kgm. less than those reported.

SUMMARY

Values were obtained in barbitalized dogs for the bleeding volume, the pre-hemorrhage circulating blood volume, the volume recruited during hemorrhage, and the liminal circulating blood volume. After control values had been obtained for each animal by a nearly maximal non-fatal hemorrhage, the drawn blood was completely re-injected. Final values were obtained four hours later by bleeding the animals to death. Blank experiments were done in which the animals were undisturbed during the four-hour interval. In these the final values were generally in good agreement with their controls. No large changes in bleeding volume were observed. Fairly large reductions in the liminal circulating volume were always associated with nearly equal reductions in the pre-hemorrhage volume. The rate of blood recruitment on the final bleeding was about 75 per cent of the rate on the control bleeding, without much change in the calculated hematocrit for the recruited blood.

When shock-like states were induced during the last three hours of the interval by continuous intravenous injection of epinephrine, by intraperitoneal injection of isotonic glucose solution, or by intestinal strangulation, final values quite different from their controls were obtained. Bleeding volume was reduced to about 50 per cent of its control value by all three procedures, the average decline being about 29 cc./kgm. In epinephrine shock about half the loss of bleeding volume was attributable to an elevation of the liminal circulating volume, the remaining half to a lack of blood recruitment during hemorrhage. In shock from intraperitoneal glucose approximately one-third of the loss of bleeding volume was attributable to each of these two factors, the remaining one-third to a decline in the pre-hemorrhage volume. In shock from intestinal strangulation, decline in the pre-hemorrhage volume was responsible for about three-fifths of the loss of bleeding volume, the remaining two-fifths being due to a lack of blood recruitment during hemorrhage, with an insignificant contribution from elevation of the liminal volume. The rate of blood recruitment on the final bleeding, as well as its absolute volume, was reduced in all the shock studies in excess of the reduction found in the blank experiments. In epinephrine shock, and in

intestinal strangulation, no cells appeared to be recruited to the circulation during the final hemorrhage.

No constant relationship was found, for all three types of shock, between the loss of bleeding volume and either the loss of circulating (pre-hemorrhage) plasma volume, or the decline in mean arterial pressure (pre-hemorrhage).

The rate of disappearance of the dye T-1824 appears to be increased in all three types of shock, as compared with the rates found in the blank experiments. The error from this source in our final values for shocked animals does not appear to necessitate revision of any of the foregoing statements.

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RESPONSE OF THE GASTROINTESTINAL TRACT TO INGESTED GLUCOSE SOLUTIONS

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Cori (1) concluded in his classic paper that the rate of absorption of glucose was not influenced by variations in the concentration of the administered solution. On the other hand Macleod et al. (2) were led to conclude that absorption was greatest from a glucose solution of 13.5 per cent concentration. MacKay and Bergman (3) have, however, reported that the absorption rate was very definitely influenced by the concentration of the administered solutions. Numerous papers may be found in the literature in support of each of these concepts. Cori (1) cited some evidence that the absorption coefficient (grams of glucose absorbed per 100 grams of body weight per hr.) was constant over a period of time, in other words that the rate of absorption was constant. Objection was made to this by Pierce et al. (4) and Feyder and Pierce (5) whose research revealed a decreased rate of absorption during the second and third hours of the absorption period. Another basic postulate underlying the Cori absorption coefficient is the linear relationship between body weight and the rate of absorption. Numerous investigators have disagreed with this; yet the Cori absorption coefficient is used to this day by many to report results of absorption studies. In view of the wide divergence of opinion it was decided to investigate with a large number of animals many of the factors which might influence the absorption of glucose and to devise a method which permitted simultaneous study of the variations in gastric secretion and gastric emptying which accompanied changes in absorption.

METHODS. Rats fasted 48 hours (except a few groups shown in table 6 which were fasted 24 hrs.) were fed glucose solutions of known concentrations and volumes, usually 1.7 to 1.9 cc. After a definite time interval the animals were sacrificed, the alimentary tract exposed and ligatures placed at the cardiac, pyloric and ileo-cecal sphincters. The stomach was placed in an evaporating dish, slit open and the pH of its contents measured electrometrically using a micro glass electrode. The fraction of a drop of fluid contained in the electrode capillary was returned to the evaporating dish. Then 25 cc. of sodium borate solution of known concentration (N/5 to N/10) were pipetted into the dish and the contents thoroughly mixed. The solution was then filtered through coarse filter paper. Essentially the same procedure was followed with the contents of the small intestine.

[1] The data reported in this paper were taken from a thesis presented to the Graduate Council of the University of Vermont in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Physiological Chemistry.

A 5 cc. portion of the filtrate was titrated with standard N/10 sulfuric acid using methyl red as indicator. From the change in concentration of the sodium borate the volume of gastric or intestinal fluid was calculated. Another portion of the filtrate was suitably deproteinized and analyzed for glucose by the method of Shaffer and Somogyi (6), by a combination of the Bertrand and the Munson and Walker methods (7), or by a modification of the latter. Another part of the filtrate was analyzed for chloride by the method of Whitehorn (8).

The volume of fluid emptied from the stomach during the experimental period was calculated on the basis of the following principles: The rate at which glucose passed the pylorus was a function of a, the rate of flow of the solution, and b, the concentration of that solution. All three variables were functions of time, two of which were determined experimentally: the rate of passage of glucose in grams, $E = dt^m$; and the concentration of the solution being emptied, $C = at^n + b$. A differential equation was set up connecting the three variables, $V = 100dm \int \frac{t^{m-1}}{at^n + b}\, dt + k$. Solution of this equation permitted the calculation of the volume of fluid emptied from the stomach during the experimental period. To check the validity of the final equation the data of Pierce, Haege and Fenton (9) on gastric emptying (obtained directly by cannulation of the duodenum just below the pylorus) were employed, and the calculated value of the volume of fluid emptied was compared with that actually observed. The agreement was very good. Calculations made by Holtz and Schreiber (10) could be used for the same purpose and yielded similar results. These workers, however, made the assumption that the concentration of a glucose solution decreased linearly with time, an assumption which is not justified.

Since the volume of injected solution, the residuum, the volume of fluid remaining in the stomach at the end of the experimental period and volume of solution emptied from the stomach during that period were all known, it became possible to compute the volume of gastric secretion. In similar fashion the net intestinal secretion could be calculated.

Objection may be raised to the use of forced feeding in absorption studies. However, voluntary feeding proved unsatisfactory in this work because of the short time intervals involved and because of the intention to administer definite predetermined amounts of glucose.

Since in the calculation of the volume of chyme emptied from the stomach the assumption was made that sugar left the stomach only by way of the pylorus, it was necessary to determine the extent of gastric absorption of the sugar. Under Nembutal anesthesia ligatures were placed at the cardia and pylorus of each of a group of white rats fasted 24 hours. Two cc. of 50 per cent glucose were injected directly into the stomach with syringe and needle. At the end of a time interval varying from 17 to 60 minutes the stomach was excised, its contents washed into a volumetric flask, the proteins precipitated and the sugar content determined. In some experiments the method of Cori (1) was used.

Results: The 13 animals used for the gastric absorption studies ranged in weight from 155 to 280 grams and were fed between 933 and 1015 mgm. of

glucose. The amount of glucose not recovered and presumably absorbed ranged from 0 to 45 mgm. with an average of 18 mgm. It is perhaps significant that when the gastric mucosa was slightly crushed during the washing process, recovery of the administered sugar was much more complete.

The data in table 1 show the effect of concentration upon the various factors studied. After some preliminary work had been done on male and female animals of colony A (series B, A, F, C, E, N, M and D), it was decided to study

TABLE 1

Absorption period 60 minutes

SERIES	PRELIMINARY STUDY, COLONY A, MALES AND FEMALES								COLONY B, MALES			
	B-60	A-60	F-60	C-60 AB	E-60 AB	N-60 AB	M-60 ABR	D-60 AB	III-A	III-B	III-C	III-D
Number of animals	2	3	12	15	20	18	35	24	24	24	22	22
Solution fed												
Concentration—%	0	5.5	10	13.5	25	40	50	65	25	40	50	65
Glucose—mgm.	0	103	173	239	410	694	857	1146	448	701	883	1141
Body weight—gram	270	272	209	174	191	177	190	156	225	239	235	236
Gastric contents												
Fluid vol.—cc.	0.32	0.53	0.66	1.03	1.76	2.49	3.05	2.79	1.65	2.46	2.59	3.12
pH	2.9	2.8	4.3	3.4	4.2	4.8	5.1	5.7	3.4	4.7	5.0	5.3
Glucose—mgm.	0	2	6	30	138	352	470	625	168	384	511	755
Chloride—mgm.	2.3	2.5	2.3	2.5	4.4	6.6	7.5	7.7				
Gastric secretion—cc.		2.17	2.09	2.16	1.78	1.86	2.35	2.06	1.27	1.62	1.62	1.97
Gastric emptying												
Glucose—mgm.	0	101	167	209	272	343	387	521	280	317	373	386
Stand. error									10.7	12.2	16.9	16.1
Chyme—cc.		3.95	3.63	3.33	2.10	1.55	1.46	1.47	1.85	1.35	1.24	1.05
Intestinal contents												
Fluid vol.—cc.	0.81	1.31	1.20	1.71	2.63	2.16	3.20	3.51	3.09	3.34	3.29	3.10
Glucose—mgm.	0	5	10	23	56	68	109	155	40	55	88	75
Chloride—mgm.	3.2	5.2		4.7	6.2	6.2	7.4	6.8				
Intestinal secretion—cc.		−4.05	−3.84	−3.03	−0.88	−0.80	+0.33	+0.63	−0.17	+0.58	+0.64	+0.64
Absorption—mgm.	0	96	156	188	216	275	278	366	240	262	285	310
Stand. error									11.9	11.6	14.5	16.1
Absorption coefficient		36	82	109	114	161	152	235	109	112	123	137

on each working day the same number of animals from each of the series under investigation in order to rule out the influence of temperature changes, minor differences in routine, etc. In this manner results for series III-A, B, C, and D (male rats of colony B) were obtained. Since it seemed virtually impossible to determine the absolute volume of fluid poured into the intestine from sources other than the stomach, only the net intestinal secretion was calculated. This represents the balance between the addition of fluids other than chyme and the removal of fluid by absorption and by emptying into the cecum.

Individual results from the nearly 1000 animals used in this study are not shown here, but examination of these data (11) showed that within any given series the concentrations of chloride and glucose in the recovered fluids were nearly constant. The Cori absorption coefficient was subject to great variations even among animals of nearly the same body weight and fed the same concentration of glucose solutions. There was a close relationship between gastric emptying and intestinal absorption. In one series of 24 animals a correlation coefficient of 0.8 was found between the amount of glucose emptied and the amount absorbed.

TABLE 2

Preliminary study, colony A, males and females

SERIES	X	A-15	A-30	A-45	A-60	F-15	F-30	F-45	F-60	C-15	C-30	C-45	C-60
Absorption period—min.		15	30	45	60	15	30	45	60	15	30	45	60
Number of animals	25	10	10	3	3	10	10	11	12	15	15	15	15
Solution fed													
Concentration—%		5.5	5.5	5.5	5.5	10	10	10	10	13.5	13.5	13.5	13.5
Glucose—mgm.		103	104	103	103	178	177	176	173	237	237	238	239
Body weight—gram	190	216	239	253	272	176	232	200	209	187	180	182	174
Gastric contents													
Fluid vol.—cc.	0.44	1.16	0.80	0.43	0.53	1.70	1.14	0.78	0.66	1.62	1.56	1.43	1.03
pH.	4.1	4.0	3,9	5.1	2.8	3.4	4.7	4.7	4.3	3.7	3.6	3.9	3.4
Glucose—mgm.		20	11	1	2	56	31	14	6	105	80	40	30
Chloride—mgm.	2.1	3.0	2.8	2.9	2.5	3.2	2.9	2.6	2.3	2.7	3.2	3.2	2.5
Gastric secretion—cc.		1.35	1.51	1.64	2.17	1.60	1.63	1.70	2.09	1.06	1.63	2.12	2.16
Gastric emptying													
Glucose—mgm.		83	92	102	101	122	146	162	167	132	157	199	209
Chyme—cc.		2.50	3.02	3.52	3.95	2.10	2.69	3.12	3.63	1.64	2.27	2.89	3.33
Intestinal contents													
Fluid vol.—cc.	1.41	1.52	1.27	1.28	1.31	1.84	2.10	1.65	1.20	1.95	1.86	2.20	1.71
Glucose—mgm.	3.0*	9	6	4	5	19	18	18	10	40	33	28	23
Chloride—mgm.	4.4	4.4	4.1	5.2	5.2					5.4	5.4	5,1	4.7
Absorption—mgm.		74	86	98	96	103	128	144	156	92	124	171	188
Absorption coefficient.		141	76	52	36	238	114	98	82	205	146	127	109

* Reducing substances expressed as glucose.

Series X (table 2) consisted of a group of animals which were fasted 48 hours and then sacrificed without being fed. In this way the contents of the rats' stomachs and intestines prior to feeding glucose solutions were established. Distilled water was fed to 15 animals of series B (only B-60 shown in table 1). It was clear that water was emptied very rapidly from the stomach and that within 15 minutes after feeding the chloride and water content of the gastrointestinal tract had returned practically to the prefeeding level. The results with glucose solutions of 5.5, 10 and 13.5 per cent concentration are shown in table 2, those with solutions of 50 and 65 per cent concentration in table 3. In these

studies animals of colony A were used. In table 4 are shown the results obtained with male rats of colony B. In these experiments 50 per cent glucose was used exclusively. On any working day one animal was used from each of series IV-A, B, C and D to rule out the effects of changes in temperature, etc. Series IV-E, F, G, H and I, dealing chiefly with absorption, were carried out by the Cori method (1).

TABLE 3

Preliminary study, colony A, males and females

SERIES	M-15 A	M-30 A	M-45 A	M-60 A	M-120	M-180	M-15 B	M-30 B	M-45 B	M-60 BR	D-15	D-30	D-45	D-60 A
Absorp. period—min.	15	30	45	60	120	180	15	30	45	60	15	30	45	60
Number of animals	10	10	9	10	9	10	15	15	14	25	14	14	12	24
Solution fed														
Concentration—%	50	50	50	50	50	50	50	50	50	50	65	65	65	65
Glucose—mgm.	850	850	850	853	850	850	842	842	842	858	1164	1181	1147	1164
Body weight—gram	183	207	193	194	171	158	203	191	197	188	177	177	191	169
Gastric contents														
Fuid vol.—cc.	2.49	2.77	2.51	2.94	2.86	2.17	2.91	3.01	3.19	3.09	2.32	2.44	2.82	2.54
pH	4.4	4.1	5.8	4.6	3.1	4.1	5.5	5.8	4.2	5.6	5.5	5.4	5.6	5.7
Glucose—mgm.	525	532	493	453	393	221	569	572	544	477	593	619	539	467
Chloride—mgm.	5.3	6.1	6.9	7.8	7.2	6.5	7.0	7.4	7.8	7.4	5.6	6.0	7.1	7.0
Gastric secretion—cc.	1.37	1.84	1.77	2.36	2.79	3.81	1.85	2.04	2.38	2.44	1.35	1.78	2.48	2.40
Gastric emptying														
Glucose—mgm.	325	318	357	400	457	629	273	270	298	381	571	562	608	697
Chyme—cc.	1.02	1.21	1.40	1.56	2.07	3.28	1.07	1.16	1.32	1.51	1.26	1.60	1.87	2.09
Intestinal contents														
Fluid vol.—cc.	3.12	3.02	2.00	2.66	2.29	2.68	3.62	3.58	3.15	3.41	2.62	2.66	2.69	3.59
Glucose—mgm.	127	108	71	82	60	75	132	98	102	119	151	158	128	197
Chloride—mgm.	6.8	6.7	5.9	6.5	5.6	6.2	8.2	8.0	7.1	7.7	6.4	6.4	6.4	6.7
Absorption—mgm.	198	211	286	318	397	554	141	172	195	262	430	411	479	499
Absorption coeff.	434	211	198	169	119	120	291	188	136	145	1019	479	347	309

The data in tables 5 and 6 show the relationship of age, body weight and sex to the response of the gastrointestinal tract. Series I-A and I-B were carried out at the same time while I-C and V-A followed a little later. The values shown in table 6 were determined by the Cori method. All animals came from colony B. The animals in series V-F (table 6) emptied 341 mgm. of glucose from the stomach during the hour while those in series VI-B emptied 414 mgm. These two values are not shown in the table.

Discussion. In agreement with Maddock, Trimble and Carey (12) no ap-

preciable glucose absorption from the stomach could be found. Macleod, Magee and Purves (2) have reported similar findings, while others, working with dogs and humans, have reported significant absorption of glucose from the stomach when concentrated glucose solutions were administered.

A. *Effect of concentration.* The finding of increased intestinal absorption from the more concentrated solutions has been shown to be statistically significant and is in direct contrast to the data of Cori (1). It does agree with the

TABLE 4

Results obtained with male rats of colony B

SERIES	IV-A	IV-B	IV-C	IV-D	IV-E	IV-F	IV-G	IV-H	IV-I
Absorption period—min	15	30	45	60	15	30	45	60	90
Number of animals	24	24	24	24	10	8	7	17	16
Solution fed									
Concentration—%	50	50	50	50	50	50	50	50	50
Glucose—mgm	887	886	886	887	943	900	886	896	897
Body weight—grams	187	191	180	180	305	290	271	283	282
Gastric contents									
Fluid vol.—cc	2.57	2.85	2.74	3.06					
pH	5.1	5.7	5.5	4.9					
Glucose—mgm	653	642	579	594					
Gastric secretion—cc	1.02	1.46	1.57	1.89					
Gastric emptying									
Glucose—mgm	234	245	307	293				260	397
Stand. error—mean	12.6	7.7	12.4	11.5					
Chyme—cc	0.66	0.82	1.04	1.04					
Intestinal contents									
Fluid vol.—cc	3.61	3.43	3.41	2.94					
Glucose—mgm	126	98	108	88					
Absorption—mgm	106	148	200	204	121	176	201	240	346
Stand. error—mean	6.2	5.6	10.1	6.9	9.0	14.0	6.6	10.5	15.1
Absorption coefficient	233	158	151	112	160	122	99	86	83

results of MacKay and Bergman (3) and others. The Cori absorption coefficient was not constant even where body weights were practically the same.

The fact that the pH of the gastric contents increased when the more concentrated glucose solutions were fed indicates that the acid component of the gastric juice has been progressively inhibited. This inhibition of acid production by concentrated glucose solutions has previously been shown by Manville and Munroe (13). Since, however, the total gastric secretion increased when

concentrations from 25 to 65 per cent were fed, it must be concluded that there was a marked increase in the production of the non-acid component of the gastric juice, due very likely to the increasing osmotic pressure of the gastric contents.

TABLE 5

Results obtained with rats of colony B; absorption period 60 minutes

SERIES	I-A	I-B	I-C	V-A
Number of animals	25	25	25	24
Solution fed				
Concentration—%	50	50	50	50
Glucose—mgm	882	883	897	882
Sex	M	M	M	F
Body weight—grams	246	129	182	183
Age—days	149	68	127	163
Length of fast—hours	48	48	48	48
Gastric contents				
Fluid vol.—cc	3.43	3.09	2.83	3.29
pH	5.4	5.2	5.6	5.2
Glucose—mgm	557	605	571	510
Gastric secretion—cc	2.44	1.84	1.72	2.50
Gastric emptying				
Glucose—mgm	325	279	326	371
Stand. error—mean	10.5	11.1	14.8	13.0
Chyme—cc	1.21	0.96	1.12	1.41
Intestinal contents				
Fluid vol.—cc	3.18	2.15	2.53	3.72
Glucose—mgm	65	55	76	85
Intestinal secretion—cc	+0.56	−0.22	0	+0.90
Absorption—mgm	261	224	250	286
Stand. error—mean	10.5	12.5	9.7	12.7
Absorption coefficient	107	175	137	158

The progressive decrease in the volume of chyme emptied during the experimental period as more and more concentrated solutions of glucose were fed is a quantitative expression of the well known fact that concentrated glucose solutions in contact with the duodenum inhibit gastric motility as shown by Quigley and Phelps (14) and others. The emptying of the stomach was inversely

proportional to the square root of the concentration fed. Because the glucose concentrations of the solutions leaving the stomach increased as greater concentrations of sugar were fed, the amount of glucose reaching the small intestine increased despite the decrease in the volume emptied. It seems likely that this increased amount of sugar available in the intestine determined in part the increased amount of sugar absorbed. The increasing amount of net intestinal secretion may be attributed to the greater osmotic pressure of the intestinal contents as more and more concentrated glucose solutions were fed.

TABLE 6

Results obtained with rats of colony B; absorption period 60 minutes; concentration of solution fed 50%

SERIES	NO. OF ANIMALS	BODY WEIGHT	AGE	SEX	LENGTH OF FAST	GLUCOSE FED	GLUCOSE ABSORBED	ABSORPTION COEFFICIENT
		grams	*days*		*hours*	*mgm.*	*mgm.*	
II-A.....	24	204		M	48	933	259 ±7.5*	128
V-B.....	24	160	147	F	48	957	242 ±7.6	153
II-B.....	30	116		M	48	966	220 ±6.1	192
II-I.....	9	116	51	M	48	849	203 ±8.5	175
V-E_1.....	6	96	51	F	48	849	177	185
II-J.....	8	152	60	M	48	842	223 ±14.3	144
V-E_2.....	3	108	61	F	48	838	187	173
II-K.....	4	181	70	M	48	855	242	132
V-E_3.....	3	120	70	F	48	855	182	151
II-L.....	6	168	80	M	48	833	233	141
II-N.....	11	181	138	M	48	976	257 ±13.4	144
V-D.....	8	160	127	F	48	885	219 ±14.0	137
II-O	10	270	184	M	48	876	252 ±14.5	93
II-D.....	24	145	73	M	48	873	220 ±13.9	152
II-E.....	20	225	152	M	48	873	204 ±12.0	91
V-C.....	7	168	152	F	24	867	258 ±13.7	154
VI-A....	7	259	152	M	24	867	348 ±10.5	135
V-F.....	25	156		F	24	994	290 ±8.9	187
VI-B....	24	232		M	24	994	378 ±14.1	165

* Standard error of the mean.

B. *Relation of time.* The finding that the gastric emptying rate was greatest during the 15 minutes following feeding was in good agreement with the data of Pierce, Haege and Fenton (9) obtained by an entirely different method. The virtually complete cessation of gastric emptying during the second quarter hour when concentrated solutions were used was in nice agreement with the observations of Quigley and Phelps (14) and Greengard, Gray and Ivy (15). As Karr et al. (16) have shown to be true in humans, the intragastrically administered glucose solutions begin to be emptied from the stomach almost immediately upon being placed there. This early burst of activity may then be pictured as being followed by inhibition of nervous and hormonal origin; in due time, as the inhibitory phase wears off, gastric emptying is gradually

resumed but never reaches the initial rapid rate. It can be seen from table 3 that of the total amount of sugar emptied in three hours more than half was poured out in the first 15 minutes. Of particular interest was the apparent second inhibitory phase of gastric emptying observed in the first experiments in table 3 (M-15-A to M-180). During the entire second hour of the experiment only 57 mgm. of glucose were emptied or an average of 14 mgm. per quarter hour as compared with 43 mgm. emptied during the last quarter of the first hour. In the first experiments of table 4 (IV-A, B, C and D) a similar situation was seen. In this case the periods of inhibition were during the second and fourth quarter hours. From this it would seem that when gastric emptying was resumed after the first inhibitory phase enough chyme of a sufficiently high glucose concentration was emptied to initiate a second phase of inhibition of gastric motility.

The rate of gastric secretion decreased with time as might be expected. The rapid dilution of very concentrated glucose solutions observed in these studies agreed well with the finding of Ravdin et al. (17) that a 50 per cent glucose solution placed in the dog stomach was reduced to 17.9 per cent in one hour. Most significantly the rate of absorption, while irregular, did essentially decrease with time. It could well be argued that the emptying and absorption rates decreased with time in the studies shown in table 2 because not enough glucose was present to maintain a constant rate. This objection cannot be made to the experiments in which 50 and 65 per cent glucose solutions were fed. Actually at the end of three hours (series M-180) 296 mgm. of glucose still remained unabsorbed. Contrary to Cori's concept (1) the rate of absorption as well as the absorption coefficient decreased with time. This finding agrees well with those of a number of investigators (3, 4, 5).

C. *Relation of body weight, age and sex.* To prove his theory that absorption was proportional to body weight, Cori (1) cited particularly data of two animals: the first a female rat weighing 117.7 grams and absorbing 219 mgm. of glucose in one hour, the second a male weighing 173.7 grams and absorbing 320 mgm. These values expressed as grams of glucose absorbed per 100 grams of body weight per hour were 0.186 and 0.184 respectively. The chief objection to this seemingly excellent proof lies in the fact that the light animal was fed 522 mgm. of glucose, while the heavier received 1145 mgm. Since absorption has been shown to vary with the amount of glucose administered and since the absorption rate decreases with time, the absorption coefficient cannot be used as a reliable means of reporting absorption data. Furthermore, it can be seen from tables 5 and 6 that absorption did not vary linearly with body weight. Neither was absorption entirely independent of body weight. It may be concluded that male animals weighing more than 145 grams and 70 or more days old absorbed about the same amount of glucose in one hour. Cori (1) did, of course, state that he intended to apply the absorption coefficient only to animals ranging in weight from 120 to 180 grams. Yet a comparison of series I-B (129 grams) with I-C (182 grams) shows the former absorbing 224 mgm. of glucose, the latter 248 mgm., a difference which was found to be statistically

significant, but one which should be much greater if absorption increased in proportion to the body weight.

It is somewhat difficult to explain the low absorption rates of series II-D and II-E. This finding could not be correlated with prevailing conditions of temperature and humidity. This points to the absolute necessity in this type of work to compare data from experiments performed simultaneously.

From table 5 it becomes apparent that the gastric emptying rate of the immature animals (series I-B) was less than of the older animals (series I-A and I-C).

Calculations on a group of 24 animals gave the following coefficients of variability: absorption in mgm. 27.5 per cent, absorption coefficient by body weight 30.1 per cent, absorption coefficient by surface area 25.2 perc ent. This agrees with the findings of Pierce et al. (4) and many others.

Although Deuel et al. (18) and Althausen (19) concluded that female rats absorbed more glucose than males, MacKay and Bergman (3) found little difference. The results in these papers were expressed in terms of the absorption coefficient (in the MacKay paper on the basis of body surface). If these results are recalculated into terms of the absolute amount of glucose absorbed, glucose absorption in the male was equal to or greater than in the female. With one exception the results of the present study bear out the findings of the earlier workers: the female animals absorbed less sugar than the males, but the absorption coefficients were the same in one case or definitely greater for the females. Only in one case (series V-A) was the absolute amount of glucose absorbed greater for the females than for the corresponding series of males. It is interesting to note that if the animals were fasted only 24 hours prior to the experiment, the difference in absorption between males and females was much more pronounced.

SUMMARY AND CONCLUSIONS

1. No appreciable glucose absorption occurred from the rat stomach ligated at the cardia and pylorus when a 50 per cent glucose solution was placed into the stomach.
2. Absorption from the intestine of the intact animal increased significantly as the concentration of the solution fed increased.
3. The volume of chyme emptied from the stomach was inversely proportional to the square root of the concentration of glucose fed.
4. The total volume of gastric secretion reached a minimum with 25 per cent glucose. The pH of the gastric contents increased with the concentration fed.
5. The rates of gastric secretion, gastric emptying and intestinal absorption decreased with time.
6. When concentrated glucose solutions were fed, the stomach showed one and sometimes two phases of inhibition of motility.
7. The rates of gastric emptying and intestinal absorption were closely related.
8. The Cori absorption coefficient was not constant.

9. Animals weighing 145 grams or more and 70 or more days old absorbed about the same amount of glucose per hour when a 50 per cent solution was fed.

10. Animals weighing less than 145 grams and less than 70 days old absorbd less glucose and had lower gastric emptying rates.

11. With one exception the females showed a lower absorption rate than male animals. This difference was much more pronounced after 24 than after 48 hours of fasting.

12. Increasing the length of the preexperimental fast from 24 to 48 hours decreased both the emptying and absorption rates.

The author wishes to thank Dr. Harold B. Pierce for his helpful suggestions and patient teaching.

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LIPOTROPIC ACTION OF LIPOCAIC[1]

A Study of the Effects of Lipocaic, Methionine and Cystine on Dietary Fatty Livers in the White Rat

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In 1930 Hershey (1) and in 1931 Hershey and Soskin (2) reported that the addition of 10 grams of lecithin daily to the diet of depancreatized dogs treated with insulin was effective in preventing the accumulation of fat in the livers of these animals and in permitting survival. In 1932 Best, Hershey and Huntsman (3) demonstrated that a diet of mixed grain and 40 per cent beef fat would produce a marked fatty infiltration of the livers of normal rats. This type of dietary fatty liver was found to be prevented by the addition of small amounts of choline to the food (4–6). The beneficial effect of lecithin in preventing fatty infiltration of the liver in depancreatized dogs was found to be due to choline (7, 8). Following this demonstration, it was widely believed that the active principle in raw pancreas, previously shown to be effective in preventing fatty infiltration of the liver of depancreatized dogs, was also choline. In 1936 Dragstedt, Prohaska and Harms (9, 10) concluded that the effect of raw pancreas feeding in preventing fatty livers and permitting survival of depancreatized dogs was not due to pancreatic enzymes or to choline but to a specific substance in pancreas. This substance they believed to be a hormone, which they called lipocaic. They based this contention on the demonstration that the amount of choline present in an effective dose of pancreas was ineffective when given in pure form and on the finding that the feeding of liver and brain, both of which contain more choline than does pancreas, was also ineffective. In a later paper (11) the preparation of an active extract of pancreas almost free of choline was described.

In 1937 MacKay (12) reported that an extract of pancreas prepared as described by Dragstedt, Van Prohaska and Harms was effective in preventing and in curing the type of fatty infiltration of the liver that occurs in rats on a low protein, high fat diet. This finding was confirmed by Aylward and Holt (13) and by Best and Ridout (14). Both groups and also MacKay and Barnes (15) however concluded that the effect of this pancreas extract could be accounted for on the basis of its choline and protein content. On the other hand Channon, Loach and Tristam (16), using similar methods, came to the opposite conclusion. In commenting on this work Dragstedt and his associates (11) pointed out that fatty infiltration of the liver in animals may be produced in a great variety of ways and that the significance of each state may not be the same. Subsequent work has demonstrated that a number of quite different factors may influence

[1] This work has been aided by grants from Armour and Company, The Josiah Macy Jr. Foundation and the Douglas Smith Foundation for Medical Research of the University of Chicago.

the fat content of the liver of normal animals fed various dietary modifications and both McHenry (17) and Best and Lucas (18) have called attention to the necessity of distinguishing various types of fatty livers which have been utilized in experimental studies.

Methionine has been demonstrated to be a potent lipotropic substance (19–21). Supplementary methionine has been reported to prevent and cystine to augment deposition of fat in the livers of rats on a high fat low protein diet (22–25). The marked lipotropic action of certain proteins has been correlated with their methionine content (26, 27). These observations indicate the possibility that the lipotropic activity of lipocaic may be due to the presence of methionine. Inositol, recently reported as a potent lipotropic factor (28, 29), has also been suggested as the active substance in lipocaic. When tested on depancreatized dogs, however, (34) it was found to be less effective than an equal amount of fat-free pancreas extract. Since this extract could not be pure inositol, its activity must have been due to other substances.

TABLE 1

Showing the effects of methionine, cystine and lipocaic on dietary fatty livers of white male rats

DIET	NO. RATS	AVERAGE INITIAL WT.	AVERAGE WT. CHANGE	AVERAGE FOOD PER DAY	* AVERAGE TOTAL LIVER FATS
		grams	*grams*	*grams*	*% Std. error*
Basal	8	133.0	−4.7	8.0	31.52 ±3.052
0.5% cystine	4	124.0	+0.5	6.8	29.17 ±2.249
0.5% methionine	8	130.0	+19.6	9.0	9.35 ±1.033
0.5% cystine, 0.5% methionine	8	121.0	+18.9	8.6	10.43 ±1.434
1.25% lipocaic #1	4	126.0	+6.0	8.2	7.39 ±1.370
3.0% lipocaic #2	4	118.0	+17.7	10.7	6.20 ±1.154
1.25% lipocaic #1, 0.5% cystine	2	114.0	+17.0	9.5	7.78 ±1.465
5.0% lipocaic #2, 0.5% cystine	4	113.0	+44.0	11.8	6.86 ±0.425
1.6% lipocaic #3	4	199.0	−15	11.2	6.01 ±1.004
0.3% lipocaic #3	8	193.0	−11.0	11.2	15.61 ±1.759
0.1% methionine	8	198.0	+8.1	11.5	26.49 ±4.210

Fatty livers produced in rats by feeding high fat, low protein, choline deficient diets are characterized by marked increase in the glyceride and relatively small

increase in the cholesterol fractions of liver lipids. The same is true of the fatty livers obtained by feeding or injecting rats supplementary thiamine, with or without fat in the diet. These have been variously termed "thiamine" or "dietary" or "fat" fatty livers, and are readily prevented by small amounts of choline and protein (or methionine). According to McHenry, on the other hand, fatty livers obtained in rats by feeding or injecting supplementary biotin contain large amounts of cholesterol and relatively smaller amounts of glycerides. In this respect they are similar to the fatty livers occurring in depancreatized dogs maintained on insulin. Both are relatively insensitive to choline but are readily prevented by small amounts of lipocaic.

The following experiments were undertaken with a two-fold purpose: first, to determine whether choline and protein (or methionine) are entirely responsible for the lipotropic action of lipocaic in the dietary fatty liver of the white rat; and second, to investigate the possibility of substituting the white rat for the much more costly depancreatized dog in assaying various lots of lipocaic (previously suggested by McKay) (30).

Experimental. White male rats kept in individual cages were used throughout the experiment. They were observed daily and given food and water as needed. The basal diet consisted of 5 per cent casein, 40 per cent lard, 48 per cent glucose, 5 per cent Osborne and Mendel salt mixture and 2 per cent agar. Approximately 1.0 gram of Mead's Brewer's yeast powder and 4 to 6 drops of cod liver oil were mixed thoroughly with each 20.0 grams of food. The various test substances were substituted for an equivalent amount of glucose in the test diets. The only significant amount of choline was that contained in the yeast powder. No exact figures of the choline content of this particular yeast are available, but according to work by Fletcher (31), it is probably in the neighborhood of 2.4 mgm. per gram of yeast.

Three different lots of lipocaic, prepared according to directions of Dragstedt (32) were used: Raw beef pancreas was ground in the food chopper and stirred for twenty-four hours with sufficient 95 per cent alcohol (acidified with H_2SO_4) to give a final alcoholic concentration of 60 per cent, assuming that the pancreas was 60 per cent water. This was filtered through fine mesh gauze, the solids re-extracted with 60 per cent acidified alcohol for twenty-four hours, filtered, and this second filtrate added to the first. The resulting cloudy liquid was filtered through paper in the refrigerator two or three times to remove fat, brought to pH 7.0 with dry calcium oxide, and the precipitate removed by filtering through paper at room temperature. In lot 1, this final clear, brownish solution was lyophilized, and the yield was small (0.22 gram from 100 grams raw pancreas). Lots 2 and 3 were obtained by evaporating the final solution to dryness on trays in a warm chamber. The material was harvested by scraping and stored in the ice box until used. Lot 2 gave a yield of 3.1 grams and lot 3 a yield of 1.6 grams per 100 grams raw pancreas. Lot 2 was analyzed for choline[2] and methionine[3]. No choline was found. The methionine content was found to be 1.25 per cent and

[2] Analysis done by Dr. W. W. Scott, Department of Urology.

[3] Analysis by Dr. Fred C. Koch of Armour and Company.

1.28 per cent on two determinations. Lots 2 and 3 contained 10.4 and 9.22 grams per cent total nitrogen respectively.

At the end of the experimental period (21 days) each animal was weighed, etherized and the entire liver removed while the heart was still beating. The livers were freed of blood as much as possible by blotting with filter paper, placed in weighed flasks, minced, and weighed. They were then placed in a 70° oven until thoroughly dry (4–7 days), cooled in a desiccator and re-weighed.

Determination of total fats and lipids was done according to the method employed by Eichelberger (33). This is, briefly, as follows: The dried livers were extracted with ether (three times) and petroleum ether (two or three times) until fat-free, dried in a desiccator and re-weighed. The weight lost during the extraction was regarded as the weight of the total fats and lipids.

Results. The results are given in table 1. Livers of rats on the basal diet contained a very high percentage of fat.

The addition of 0.5 per cent cystine to the diet did not result in a significant change in the percentage of fat. However, three of the eight animals on the cystine diet died on the 8th, 12th and 18th days, respectively, while a fourth became obviously sick and ate nothing during the last five days of the experiment. These four animals are not included in the tabulated results.

The addition of 0.5 per cent methionine to the diet reduced the average total fats and lipids to 9.35 per cent. This does not differ significantly from the 10.43 per cent average in the group receiving 0.5 per cent methionine and 0.5 per cent cystine.

Six groups of animals received lipocaic. All animals receiving lipocaic with or without cystine had liver fats significantly lower than those fed the basal diet with or without added cystine. There was no significant difference in the percentage of fat in the livers of animals receiving lipocaic alone and of those receiving lipocaic plus 0.5 per cent cystine. The average total fats and lipids for the eighteen animals receiving adequate amounts of lipocaic (1.25 to 5.0 per cent) with or without cystine was 6.72 ± 0.467 per cent. This is significantly lower than the 9.35$\pm$ 1.033 per cent average for the eight animals receiving 0.5 per cent methionine. Furthermore, a group of eight rats fed 0.3 per cent lipocaic no. 3 had liver fats significantly lower than those fed the basal diet or with 0.5 per cent cystine. This amount of lipocaic was not as effective as 0.5 per cent methionine, however.

A group of eight rats fed the basal diet supplemented with 0.1 per cent methionine had an average of 26.49 per cent total liver fats and lipids. The difference between this and the 31.52 per cent and 29.17 per cent averages for rats receiving the basal diet with or without 0.5 per cent cystine is of dubious significance. This amount of methionine is, roughly, twice the amount of methionine contained in the 3 per cent lipocaic diet prepared with the same lot of lipocaic on which methionine determinations were made.

Discussion. We have assumed that the fatty livers obtained in this experiment belong to the type termed "dietary" fatty livers, although we have not yet determined the relative amounts of cholesterol and glycerides. It may be well

to point out that our basal diet contained some choline and all members of the B complex of vitamins (contained in the brewer's yeast powder). There is a growing body of evidence that certain members of the B complex have a profound influence upon fat metabolism and conflicting results in experiments upon lipotropic factors may, therefore, be due to slight variations in the amounts of these vitamins contained in the experimental diets used by various workers.

The basal diet in the above experiments contained only 5 per cent protein. If the nitrogen in the lipocaic were all protein nitrogen, the maximum protein added in any group was 3.15 per cent (in the group receiving 5 per cent lipocaic plus 0.5 per cent cystine). This still leaves the total protein content of the diet far below the 15 to 20 per cent which previous investigators have found necessary before a lipotropic effect is apparent when casein is the protein. Since there was no choline and less than 2 per cent methionine in the lipocaic assayed for these substances, the marked lipotropic effect of the lipocaic must have been due to some constituent other than choline, protein or methionine.

No antagonism was demonstrated between methionine and cystine or between lipocaic and cystine at the levels used in our diets. However, supplementary cystine apparently made it more difficult for animals to survive on a low protein diet. The only animals dying in the course of the experiment were three that received cystine.

CONCLUSIONS

1. Fatty livers were produced in rats by diets rich in fat and low in protein.
2. These fatty livers could be prevented by the addition of 0.5 per cent methionine or of 1.25 to 5.0 per cent pancreas extract (lipocaic).
3. The addition of 0.5 per cent cystine to the diet produced no significant effect on liver fat.
4. Since there was no choline and less than 2 per cent methionine in the pancreas extract it is concluded that the lipotropic effect of lipocaic on dietary fatty livers in rats must be due to some constituent other than choline, methionine, or the non specific action of protein.

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OBSERVATIONS IN TOTAL BILIARY FISTULA DOGS WITHOUT BILE THERAPY

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The belief that bile is essential for life is well-founded and is supported by a large volume of evidence. Whipple (1) in a review on bile in 1922 stated that bile is necessary for normal life and even actual continuation of life beyond a few months. The review by Schmidt (2) a few years later presented the same opinion. More recently, in his book on bile, Horrall (3) concluded that bile is essential for life of both man and experimental animals.

The outstanding findings in dogs which result from prolonged bile loss are spontaneous bleeding from prothrombin deficiency, osteoporosis and duodenal ulcers, as reported by Hawkins and associates (4, 5). However, Crandall et al. (6–9) in their studies on dogs with total bile fistulae reported that their animals remained alive for over a year in some cases. The dogs received no bile, but were given the fat-soluble vitamins parenterally. A hyperchromic macrocytic anemia was shown to develop in less than 4 months under these conditions. Furthermore, the anemia was responsive to liver extract therapy, and was considered to possess possibilities for assay purposes. The present report deals with total bile fistula dogs which were given no bile at any time. In spite of this, apparently normal health has been maintained over a period of years.

METHODS. Bile fistulae of the cholecystnephrostomy type were performed in 4 dogs, using the technic of Kapsinow, Engle and Harvey (10). In each case, the common bile duct was doubly ligated and cut. Previous experience showed that these animals had little appetite and lost much weight following the operation. Furthermore, they rapidly developed anemia. This situation also occurred in the 4 dogs being described. Their condition was so critical 1 month post-operatively, a decision was made to feed them by stomach tube. Feedings consisted of a quart of milk in which was thoroughly mixed 2 eggs, 50 grams of glucose, and 2 slices of white bread for each animal daily. A few days later, the dogs appeared much stronger and readily ate this same mixture from a pan. Thereafter, body weight was rapidly regained and the anemia disappeared, so this special diet was stopped after about 2 months. Since then the animals have received our stock diet for dogs, namely, canned dog food (while this was available) and dog biscuit. In addition, it was found that a quart of milk was needed daily per animal to maintain body weight. Parenteral administration of vitamins A, D and K has been continued from the time of operation to the present.[1]

[1] The animals received a daily dose of 1000 units of vitamin A, 500 units of vitamin D, and 1.1 mgm. of 2-methyl-1,4-naphthoquinone. These were dissolved in cottonseed oil and given as one intramuscular injection every 2 weeks. Alpha-tocopherol was also administered at the start, but was discontinued after a few months.

No attempt has been made to give other vitamins in addition to those in the food. Ferrous gluconate was given per os for 1 month about 2 months post-operatively but was found to be unnecessary.

Complete red blood cell studies were made at weekly intervals for 18 weeks after operation. United States Bureau of Standards certified pipettes were used for erythrocyte counts. Hemoglobin level was determined by photoelectric colorimeter. Hematocrits were measured in Wintrobe tubes. In addition, x-ray pictures were taken of the long bones, and prothrombin times, blood lipid levels, serum phosphatase values, and gastric juice acidities were determined. Three dogs are now dead, 16, 25 and 42 months, respectively, after operation. Deaths were accidental and not due directly to their bile fistulae. Post-mortem findings will be discussed later. The remaining animal is alive and apparently in good health 3½ years following operation.

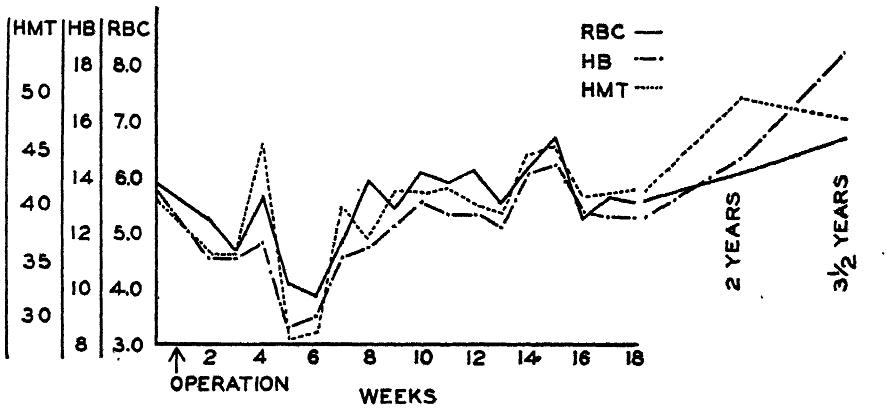

Fig. 1. Erythrocyte changes in a dog following production of complete biliary fistula. Red blood cells are expressed in millions per cu.mm., hemoglobin in grams per 100 cc., and hematocrit as percentage of whole blood volume which is cells.

RESULTS. *Erythrocyte studies.* In the first month after operation, the red cell count, hematocrit and hemoglobin fell rather abruptly. No variation in mean diameter of red cells occurred. There was no distinct change in mean corpuscular volume or mean corpuscular hemoglobin in any animal. Once the dogs were started on the special diet mentioned above, there was a rapid improvement in the blood picture with a return to normal in about 2 months. At no time was a hyperchromic macrocytic anemia observed. The results are somewhat in agreement with those of Last and Last (11), whose animals showed only an intermittent low-grade macrocytic hyperchromic anemia. Forty-two months post-operatively, the remaining 2 dogs showed a perfectly normal erythrocyte picture. The course in 1 of these dogs is illustrated in figure 1, which is typical for the other animals. In view of the relation between recovery from anemia and adequate food intake, it appears the anemia may have been a nutritional type.

Two prothrombin determinations in each of the 2 dogs which survived 3 years were 24.5, 26, 28 and 29 seconds by Link's modification (12) of Quick's method. These values are normal.

Body weight. Changes in body weight of these dogs paralleled the change in blood picture. There was a rapid weight loss in the first month followed by recovery when the dogs were given the special diet. On resumption of a regular diet plus milk, weight gain continued, 2 animals becoming several kilograms heavier than before operation. The third dog has varied in weight from somewhat above original to 1 to 2 kgm. below, usually being slightly below. The fourth animal, 5 months post-operatively, at which time body weight was greater than originally, suddenly began to lose weight. In spite of a return to the special liquid diet, this animal continued to be somewhat underweight. Following 'Seconal Sodium' (Sodium Propyl-methyl-carbinyl Allyl Barbiturate, Lilly) anesthesia, which resulted in death of this dog, necropsy revealed evidence of partial biliary obstruction which may account for the weight loss. Figure 2 shows graphically the course of weight changes in these animals.

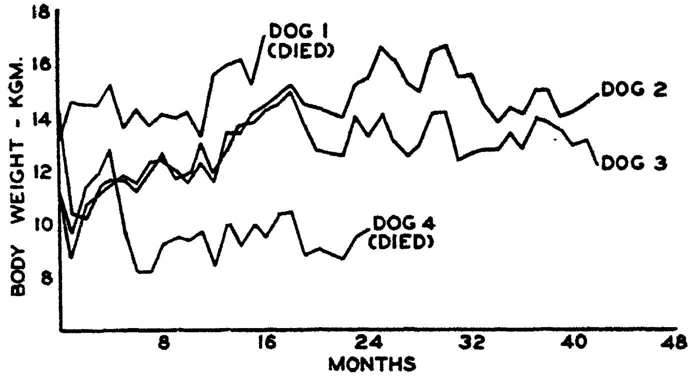

Fig. 2. Effect of complete loss of bile on body weight of 4 adult dogs. The internal bile fistula operations were performed a few days after the first weights.

X-rays of long bones. In 3 of the dogs, x-rays were taken of the long bones of of the hind limbs 24 months post-operatively. The fourth dog had died before this. A normal dog was included and exposure times were equivalent in each instance. Bone structure proved to be normal for all the bile fistula dogs, no osteoporosis being evident whatsoever. It should be remembered that these dogs received adequate amounts of vitamin D parenterally.

Blood lipid determinations. Three total blood lipid determinations[2] were performed on the 2 surviving animals 26 to 29 months post-operatively. The values obtained were 600, 650 and 1067 mgm. per 100 cc. of blood in 1 dog and 523, 480 and 597 mgm. per 100 cc. of blood in the other. These values are about at the low range of normal. Certainly no hypolipemia was present comparable to that present in depancreatized dogs or animals whose pancreatic ducts have been ligated (13).

Serum phosphatase levels. Using a modification of the method of King and

[2] All blood lipid determinations were graciously performed by Dr. L. R. Dragstedt of the University of Chicago.

Armstrong (14) adapted to a photoelectric colorimeter, alkaline serum phosphatase determinations were made in the dogs which were living 25 months post-operatively. Values ranged from 6 to 12 units which are entirely normal. This is in contrast to the report of Thannhauser and associates (15) who noted an increase of 10 to 20 times the normal serum phosphatase level in their dogs with complete biliary fistulae. They found the maximum values occurred about 1 week after operation and their animals lived an average of only 6 weeks, whereas the writer's results were found at a much later date.

Gastric acidity. Twenty-five months post-operatively, alcohol test meals were run on the 2 surviving bile fistula animals as well as 2 normal dogs. The results were entirely normal which suggests that lack of bile did not result in development of high acidity of the gastric juice. This is important in the possible etiology of peptic ulcer reported frequently to develop in total biliary fistula dogs. Dragstedt (16), however, appears to be of the opinion that hepatic infection or partial biliary obstruction may be necessary factors for development of ulcer in these animals.

Pathologic findings. Post-mortem examination of the 3 dogs now dead showed the following positive findings. In the animal which died 16 months after operation, the cholecystnephrostomy was healed, open, and functioning, the right kidney being one-half as large as the left which was hypertrophic, but otherwise normal. Mild chronic cholecystitis and cholangitis plus great dilatation of common bile and hepatic ducts were present. The 2 ligatures used to tie the common bile duct were still in place. Mild urinary cystitis also was noted. Apparently the cause of death was cellulitis of the right thigh, which may have resulted from an injection of vitamins a few days previously.

The dog which died 25 months post-operatively as a result of failure to recover from 'Seconal Sodium' anesthesia showed mild chronic cholecystitis and cholelithiasis with many small bile pigment stones in the biliary tract. The stoma between gall bladder and right renal pelvis was so small, only the tip of a probe would pass. Furthermore, the common bile and hepatic ducts were dilated to the size of a lead pencil. This dog showed additional evidence of obstruction in that the small intestines and pancreas were yellow-stained on the outside. The old ligatures of the common bile duct also were present. The left kidney was twice normal size. Around the region of anastomosis were numerous adhesions.

Death of the third animal resulted from a laparotomy performed 3½ years after cholecystnephrostomy. This second operation was done in order to make certain the common bile duct had been ligated and cut. Many adhesions were present in the operative field. In breaking these adhesions to locate the bile duct, a large hemorrhage occurred from the portal vein. In spite of treatment, the dog died 6 hours post-operatively. Necropsy revealed a normal biliary tract with little or no dilatation of the ligated common bile duct. No bile was seen in the duodenum. The opening between gall bladder and right kidney was large. One small bile pigment stone was found in the right renal pelvis. The right kidney was one-half normal size, the left kidney being hypertrophied.

Laparotomy on the fourth dog 3½ years following establishment of the fistula

showed the common bile duct to be ligated. It was dilated above the point of ligation to a diameter of about 25 mm. Incision of the dilated duct revealed it to be filled with a granular sediment which appeared to be bile pigment. Numerous adhesions were present around the fistula. The right kidney was almost completely atrophic and the left kidney about twice normal size. No other abnormality was noted.

An important negative finding was the complete absence of peptic ulcers or scars in the stomach or duodenum of these dogs. The milk in the diet may have contributed to prevention of ulcers.

Since going to press, Hawkins (17) has raised the question of vitamin E deficiency. He has noted certain changes in his bile fistula dogs that may be associated with lack of vitamin E. As the fourth dog had died of peritonitis before this problem arose, only a few tissues were available for microscopic sections. The third dog showed a small amount of brownish pigment in muscle cells of the ureter and in the phagocytes beneath the mucosa, plus some pigment in liver cells. Slight pigmentation was also noted in the gall bladder muscle of the fourth dog, while much pigment was found in phagocytes in stroma and some in the muscularis. Both of these animals at laparotomy showed a brownish discoloration on the outside of the small intestines and pancreas which was similar to that seen in the second dog. This may have been brown pigmentation of vitamin E deficiency rather than bile-staining.

SUMMARY

Four dogs with complete internal biliary fistulae lived for a considerable period of time apparently in good health without any bile therapy whatsoever. Diet consisted of dog biscuit and milk. Fat-soluble vitamins were administered parenterally. After developing anemia and losing weight immediately after operation, these animals recovered completely. Studies in some of these dogs showed normal prothrombin time, blood lipids, serum phosphatase, and gastric acidity. No spontaneous hemorrhages, osteoporosis or peptic ulcers developed. Three dogs have died 16, 25 and 42 months post-operatively, death resulting from causes other than the bile fistulae. The remaining dog is still alive and appears normal more than 3½ years after operation. The essentiality of bile for the life of the bile fistula animals appears to reside largely in its facilitating the absorption of fat-soluble vitamins.

Acknowledgment. The author wishes to express thanks to Dr. L. R. Dragstedt who offered many valuable suggestions in this work.

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X-RAY DIFFRACTION STUDIES ON FISH BONES

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For x-ray diffraction studies of bones, those of the fish are particularly well adapted. Their relatively small size and low salt content permit shorter x-ray diffraction exposures and make it possible to reduce appreciably the time necessary for decalcification. Also because of the anatomy of certain fish there are various long bones on which the functional stresses act at widely different angles. It is therefore possible in such bones to study the effects of function upon their x-ray diffraction patterns. Parallel studies were made on mammalian bones (rat, cat, lamb) in order to correlate these findings. Our x-ray diffraction apparatus has been described in former papers (Spiegel-Adolf and Henny, 1939). For this special kind of investigation a cylindrical-film camera of a 50.8 mm. radius was constructed. The specimen is rotated around its longitudinal axis, which is parallel with the slit defining the x-ray beam. The cylindrical camera was used in order to obtain the results in the form of a Hanawalt analysis. Besides this the usual flat-film cameras were used.

In a first series of experiments, we wanted to ascertain how far the diffractograms of the fish bone coincide with the ones of the mammalian bone. The latter has been widely identified with the diffraction pattern of apatite. According to references in the literature, fish bones contain more Na than mammalian bones (see Hammarsten). Hendricks and Hill suggest that the Na content of the latter is not accounted for in the assumed apatite structure. An x-ray diffraction study of the fish bone seemed, therefore, warranted.

A diffractogram of a fish rib[1] shows at least 8 diffraction lines. The most prominent in decreasing order of intensity correspond to spacings of 2.73, 1.76, 2.22 Å.

In order to obtain data directly comparable with our own results in mammalian bones and the ones of Reed and Reed on rat bones and apatite, a diffractogram of the same fish bone was made in both the flat-film and the cylindrical-film camera.

Table 1 gives a summary of our figures compared with the ones of Reed and Reed for rat bone.

A comparison of these data seem to indicate the following results:

Within a certain medium range (2.22–4.62 Å) there seems to exist a satisfactory coincidence between the diffractograms obtained in the cylindrical and in the flat camera. Because of limitations of our cylindrical-film camera, we were not able to record the diffracted rays below an appreciable angle and thus the greater atomic spacings could not be measured. Because of the longer pathway

[1] The ordinary fish rib corresponds to the lower rib of the herring and salmon.

of the diffracted x-rays in the diffracting material which occurs with the shorter spacings, the latter ones are either partially or completely missing in the diffractograms produced in the flat-film camera. The cylindrical-film camera is useful in making measurements of the intensity of the diffraction lines (as in a Hanawalt analysis) as well as in making measurements for the interplanar spacings.

2. A comparison between the data in columns 2 to 5 seems to indicate that the diffractograms of bones of fish, lamb, rat and cat are within a certain range very similar to each other. These findings are interesting in view of the small chemical differences between fish and mammalian bones mentioned before.

TABLE 1

Interplanar spacings of bones (Ångstroms)

FISH BONES		LAMB LONGIT. SECT.	LAMB CROSS SECT.*	CAT SKULL†	RAT FEMOR†	REED AND REED RAT
Circ.	Flat					
	8.62		8.31	8.62	8.62	8.29
4.63	4.62	5.16	5.16	5.16	5.16	5.31
	3.77	3.67	3.67	3.77	3.73	3.76
3.36	3.38	3.38	3.33	3.33	3.33	3.42
3.04	3.04	3.01	3.02	3.01	3.00	3.10
2.73[1]‡	2.75	2.71	2.73	2.73	2.73	2.78
		2.45				2.60
2.22[3]	2.28	2.22	2.26			2.37
						2.08
1.93		1.92	1.90			1.92
			1.82			
1.76[2]						1.71
1.43						

* Because of the orientation in longitudinal sections of bones to be discussed later a diffractogram of a cross section of a mammalian bone has been included in this table.

† Two hour exposures, while in the other diffractograms four hour exposures were used. The shorter exposure time probably accounts for the fact that the lines from the shorter spacings did not show up.

‡ Indexes in order of falling intensity.

3. In the last column of table 1, is shown data of Reed and Reed concerning the x-ray diffraction pattern of rat tibia. The agreement between these figures and ours is fairly good, in fact about as good as that between our own data. This agreement is the more interesting as Reed and Reed have been able to identify their diffraction patterns of rat bone with the ones of apatite.

In a second series of studies, x-ray diffraction patterns of decalcified bones were examined. Although J. H. Clark, for theoretical reasons, suggested the existence of crystalline organic structures within bones, no clear-cut experimental evidence has yet been offered. One of the experimental difficulties which this problem presents is due to the fact that the procedure of decalcification is able to destroy the structure of the osteo-collagen. Like most of the previous authors, we used 5 per cent HNO_3 as a decalcifying agent. By judiciously choosing the size of the

fish bones and the thinness of the bone slabs (0.25 mm.) it was possible to cut down the time of contact with the acid to two hours. The specimen was washed thoroughly in running tap water until the reaction became neutral. The swelling effect of bound acid which was possibly present and the loss of electrolytes was counteracted by keeping the decalcified and washed specimens in saline solution for about 18 hours. After this treatment the bones were dried at room temperature with or without applying a constant weight (100 grams). The diffractograms of such decalcified bones (rib of fish, longitudinal slab of femur of rat and humerus of lamb) show a distinct pattern consisting of at least two pairs of equatorial points and one pair of meridional sickles. This pattern is practically identical with the one given by tendons when the primary x-ray beam is perpendicular to the longitudinal axis of the structure (see fig. 1). A further similarity exists because stretching of tendons or decalcified bones does not result in new diffraction lines. Besides, the fact raised by Herzog and Gonell, that collagen

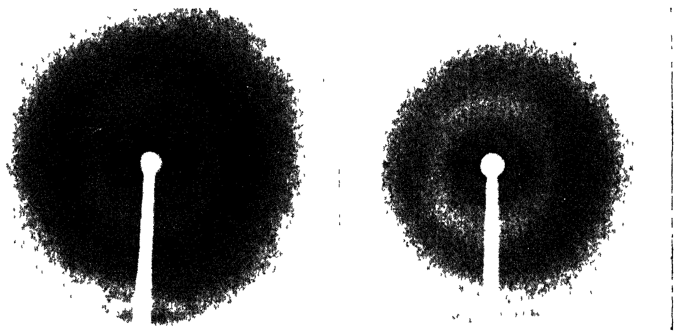

Fig. 1. a. (Left) decalcified lower rib; b. (Right) frog tendon. The longitudinal axis of each specimen coincides with the vertical axis of the diffraction pattern.

lacks species specifity seems to fit for osteo-collagen also, since decalcified bones of fish and rat give similar patterns. We are fully aware of the fact that our so-called osteo-collagen is chemically not pure collagen since nothing has been done to remove the other protein constituents (osteo albumin and osteo mucoid). Apparently in their original state they do not interfere with the diffraction pattern. Their presence becomes manifest if the fish bones have been boiled previous to decalcification. The ring corresponding to the "back bone" spacing of the proteins is sharpened, a phenomenon occurring only in proteins subject to heat-denaturation (Spiegel-Adolf and Henny). The patterns of the decalcified bones are clearly oriented and so are the patterns of longitudinal slabs of long bones (Clark and Mrgudich, Reed and Reed). We were interested in a two-fold aspect of this question. We wished to ascertain whether or not the orientation of the inorganic and the organic parts of a bone are influenced by the shape and function of the bone and if so, to what extent. For both purposes bones from herring and salmon are particularly fit. These fish have both upper and lower ribs which stand roughly at right angles (see Claus-Grobben). X-ray diffraction

patterns of longitudinal slabs of long bone (rat, lamb) and lower ribs show a marked meridional orientation in two rings corresponding to spacings 3.38 and 3.67 Å. Equatorial orientations (Reed and Reed) also could be noticed. But while Reed and Reed report a widening in the equatorial diameter of only one ring, we were able to demonstrate similar changes in at least two diffraction rings (fig. 2). Cross sections of long bones give no orientation (confirming findings of Clark and Mrgudich). But neither does the diffraction of the upper rib show signs of orientation although the primary x-ray beam intersects the bone perpendicularly to its longitudinal axis. After decalcification complete parallelism between the orientation of the original and the decalcified bones becomes manifest. No signs of orientation were noticeable either in the diffractograms of the cross section or in the one of the upper rib. In the case of the cross section, as in unstretched gelatine, the two sickles of the oriented form are replaced by a complete ring.

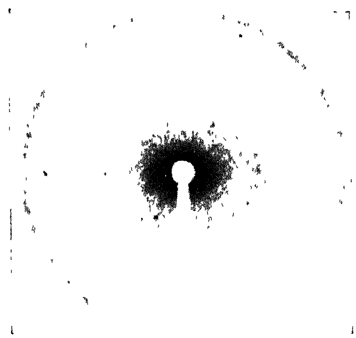

Fig. 2. Longitudinal slab of lamb humerus. The longitudinal axis of each specimen coincides with the vertical axis of the diffraction pattern.

It may be interesting to speculate as to whether the same stress has produced both the orientation in the organic and in the inorganic material pattern, or whether the former has provided the matrix for the crystallization of the inorganic salts (J. H. Clark, Bucher). We have observed a similar kind of orientation in flat skull bones of the cat which are not of cartilaginous, but of membranous origin.

The lack of orientation in both the original and decalcified upper rib of the fish seems to indicate that the function rather than the shape of the bone determines the orientation of its structure. Astbury has shown that squeezing of keratin induces an orientation similar to that produced by stretching. The orientation of the lower rib can therefore be explained by the "squeezing" action of the lateral pressure of the surroundings acting perpendicularly to the longitudinal axis. In the case of the upper rib this same pressure should act parallel to the longitudinal axis and therefore no orientation similar to the one of the lower ribs could be expected.

As an indirect proof of these assumptions, we were able to show that if a decal-

cified upper rib is dried while weighted with 100 g the subsequent diffraction pattern becomes very similar to the one of the decalcified lower rib and to the tendon pattern.

SUMMARY

1. X-ray diffraction patterns of fish bones have been recorded and compared with diffractograms of bones of various mammalians. The three most intensive diffraction lines in decreasing order correspond to spacings of 2.73, 1.76 and 2.22 A.

2. Non decalcified long bones of mammalians and lower fish ribs show marked orientation in the meridional sector of two rings. Upper fish ribs and cross section of long bones do not show signs of orientation.

3. The x-ray diffraction patterns (x-ray beam perpendicular to the longitudinal axis) of decalcified (lower) ribs of fish and of decalcified longitudinal slabs of long bones from mammalians are practically identical to those of tendon taken under similar conditions. Decalcified (upper) fish ribs and decalcified cross sections of long lamb bones show the pattern of gelatine without signs of orientation.

4. If a decalcified upper rib is stretched while drying, then a pattern similar to the one of lower ribs and tendon results.

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THE EFFECT OF DECOMPRESSION OF HUMAN METABOLISM DURING AND AFTER EXERCISE[1]

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In a previous paper (Cook, 1945) evidence was advanced which indicates a reduction in the resting oxygen consumption in humans and in mice when these organisms are subjected to severe, acute decompression. A study of cell respiration some years ago (Cook, 1931) showed the same effect to be present in mammalian and amphibian tissues, in particular muscle. It seemed therefore probable that the reactions underlying muscular activity, or exercise, would also be affected.

Six series of experiments were performed in the decompression chamber, each representing a different type and degree of muscular exertion. In each case the resting metabolism of the individual was determined (at sea level or at some specified altitude) with the McKesson respirometer. Then exercise was performed after which the recovery was followed while the subject rested. For simplicity in comparative presentation the oxygen volumes have been equated to the resting value, taking the latter as 100 per cent.

The six series were the following:

Series I. The "standard step-up" on a nine inch platform used in many of the decompression studies carried on in this laboratory. The rate was 15 steps per minute and the duration five minutes. Six subjects.

Series II. The same step-up at 20 steps per minute for ten minutes. Five subjects.

Series III. The same step-up at 15 steps per minute for twenty minutes. Five subjects.

Series IV. The subject, lying flat on his back, was required to elevate an eight pound iron bar to arms length. This was repeated at the rate of 15 times per minute for six minutes. Eight subjects.

Series V. The subject was required to perform a step-up exercise with a nine inch step. In modification A he stepped up using the *same leg* at the rate

[1] The work described in this paper was done under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of California.

of 30 steps per minute for two minutes. In modification B he *alternated legs* at the same rate and for the same duration. Nine subjects.

Series VI. The subject, while sitting, was required to grasp a thirty-five pound iron bar, lift it from a stand and hold it free for one minute, the muscular contraction being almost wholly isometric.

Series I, II, III, V and VI were performed at sea level and at 30,000 feet. Series IV was performed at sea level and at 10,000, 18,000, 30,000 and 33,750 feet simulated altitude.

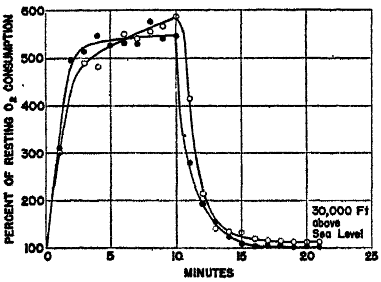

Fig. 1

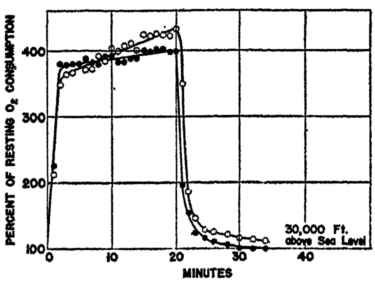

Fig. 2

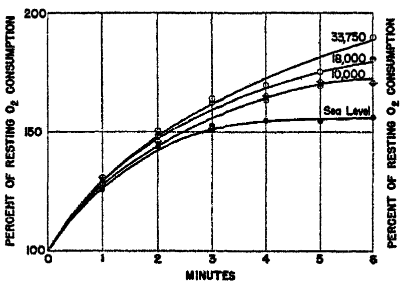

Fig. 3

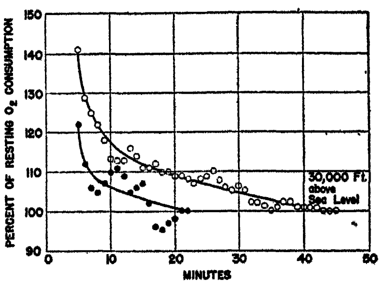

Fig. 4

● Sea level determinations.
○ Altitude determinations.

All exercises except series VI were selected such that if continued for a long enough period the subject would come to a steady state. In series I the total duration was too short for this condition to become clearly manifest. The result in series II is shown in figure 1. At sea level the mean excess oxygen usage is almost constant showing nearly, although not quite, a perfectly steady state. At 30,000 feet the excess oxygen increased more slowly during the first three minutes and then rose more rapidly until at the end of the exercise period the oxygen debt was definitely greater than it was under sea level conditions. Figure 2 illustrates the same phenomenon as found with the longer exercise of series III. The arm exercise, series IV, is even more striking (see fig. 3). This activity, for the muscular elements concerned, is far more intense and fatiguing than the standard step-up. At sea level, after approximately three minutes, a substantially steady state is attained. At 10,000 feet where, with pure oxygen

in the respirator, there can be no question of anoxia, the curve keeps rising during the six minutes. At 18,000 and 30,000 the rise is respectively more sharp. It is noteworthy, not only that the effect is observed at very low altitudes, but also that the intensity of the effect increases consistently with rising altitude.

Since the existence of a steady state during muscular activity is generally regarded as representing a dynamic balance between the anaerobic formation of acid metabolites, such as lactic acid, and their aerobic oxidation, the results here described indicate that decompression retards the latter reaction. This is in conformity with earlier published results with artificial oxidation systems which showed the site of the decompression effect to be the terminal oxidation system.

TABLE 1

SERIES	ALTITUDE	PER CENT OF TOTAL OXYGEN USED DURING EXERCISE	
I	Sea level	84.8	C.R. = 3.27
	30,000	73.5	
II	Sea level	91.7	C.R. = 6.9
	30,000	86.8	
III	Sea level	95.9	C.R. = 4.8
	30,000	90.2	
IV	Sea level	85.0	
	10,000	80.3	
	18,000	77.0	
	30,000	72.3	
	33,700	71.9	
V, one leg	Sea level	61.0	C.R. = 5.14
	30,000	45.0	
V, both legs	Sea level	63.0	
	30,000	57.0	

If oxidation is delayed one would expect to find that it finally occurs during the recovery period following exercise. Data bearing on this point are given below. In table 1 that fraction of the *total* excess oxygen which was used during the exercise is given. The balance of course was used during recovery. All values are the means for each series. If oxidation recovery is delayed or retarded then the time for complete return of the metabolism to the resting level should be greater. The data covering this point are in table 2 and figure 4 (which shows recovery in series V). In series I, II, and III it was not feasible to carry the subject to complete restoration. In those series, therefore, the data are in terms of the time to reach the value of 10 per cent above resting level. In series IV and V the times represent minutes after exercise necessary to secure complete recovery.

In the step-up series I, II and III there is a consistent and significant tendency for the oxygen consumption during exercise to be less and that after exercise to be greater at 30,000 feet than at sea level. Concurrently the final recovery, or reversion to the resting rate is materially delayed. In series IV this effect is clearly shown to be a function of altitude. One may conclude, therefore, that decompression so interferes with the aerobic removal of metabolites in the muscle (or elsewhere in the body) that there is a greater accumulation of such substances during the activity and a decelerated removal subsequently.

It would be expected that regardless of altitude the *total* excess oxygen consumption would be the same, provided, as is the case, that the work done during the exercise is identical, although the reactions might be delayed by the decompression. It appears, however, that in some cases there is an actual increase in total oxygen usage, with altitude, which means a proportionately greater energy expenditure and reduced efficiency. These data are shown in table 3. Table 3, A, gives the mean excess oxygen consumption, where the resting value is 100 per cent, for the six series. Table 3, B, shows first the total excess consumption for series IV with different altitudes; and second, the per cent increase of this excess over the sea level value.

TABLE 2

SERIES	ALTITUDE	MINUTES FOR RECOVERY SUBSEQUENT TO EXERCISE	DIFFERENCE
			min.
I	Sea level	8.5	7.5
	30,000	16.0	
II	Sea level	15.0	7
	30,000	22.0	
III	Sea level	26.0	10
	30,000	36.0	
IV	Sea level	3.3	
	18,000	4.1	
	30,000	4.7	
V, one leg	Sea level	20	22
	30,000	42	
V, both legs	Sea level	20	5
	30,000	25	

Series I, II and III show no significant change in total excess oxygen at 30,000 feet, nor does series V, using both legs. But series IV and series V, using one leg, show a very marked increase at the higher altitude. Moreover in series IV the change is linear with increasing decompression. The only discernible difference in the exercises involved is that in series IV and series V with one leg the amount

of work done by each muscle group is greater than in the standard series. In other words, the effort is localized. Series V was planned with this distinction in mind. On the one hand, a certain quantity of mechanical work is done by both legs together; and on the other hand, the same work is performed by one leg alone. The data in the tables show that under the latter circumstances the shift in per cent oxygen consumption during exercise is greater (table 1). The time of recovery is longer (table 2), and the oxygen consumption is much greater (table 3). In series IV, likewise, a small group of arm and shoulder muscles were forced to do intense work, even to the point of disagreeable fatigue. Here the same phenomena are manifest.

TABLE 3

A

SERIES	EXCESS OXYGEN CONSUMPTION DUE TO EXERCISE AT 30,000 FEET EXPRESSED AS PER CENT OF THE EXCESS AT SEA LEVEL
I	7 (Insignificant)
II	−5 (Insignificant)
III	0 (Insignificant)
IV	44 (Significant, C.R. = 4.6)
V, one leg	45 (Significant, C.R. = 3.1)
V, both legs	−7 (Insignificant)
VI	−34 (Significant, C.R. = 4.0)

B

Series IV

ALTITUDE	EXCESS OXYGEN IN PER CENT OF RESTING VALUE FOR THE PARTICULAR ALTITUDE	PER CENT INCREASE IN EXCESS OXYGEN OVER THE EXCESS AT SEA LEVEL
Sea level	340	0
10,000	418	24
18,000	462	36
30,000	488	44
33,750	523	54

In view of the results of the first five series, that of series VI appears particularly striking. In series VI the subject was forced to undergo a strenuous isometric contraction of the arm muscles in holding a thirty-five pound bar for one minute. The exercise was highly fatiguing, despite the absence of motion. As is shown in table 3, A, the excess oxygen consumption is 34 per cent less at 30,000 feet than at sea level.

The central problem posed by these results is to account for the variations in oxygen consumption during and after exercise which are induced by decompression. The tendency observed in some series for recovery to be delayed is explicable on the assumption that decompression inhibits the aerobic phase of the carbohydrate cycle. This assumption would follow reasonably from the previous demonstration of inhibition of resting metabolism. But a simple inhibition or

deceleration of the reactions as a *whole* would not predict a change in the *total* oxygen usage. The only clue to the cause of this phenomenon which has at present come to hand is the type of exercise accompanying the variation in metabolism. Exercise which is moderate and which involves free motion does not seem to alter the total excess oxygen consumption at altitude. Activity which uses a relatively small group of muscles in motion carried nearly to fatigue shows an increased consumption at altitude, but when the contraction is isometric but still fatiguing the total consumption is reduced. The essential distinction is between isotonic and isometric contraction. Two possible lines of explanation are open. 1. Decompression exerts a differential effect on the various components of the terminal aerobic oxidation system in muscle. It would have to follow that these components differ quantitatively in isometric and isotonic contraction. No concrete evidence exists to support such a hypothesis. 2. The conditions of intra-muscular circulation are different in the two types of contraction, and therefore the oxygen pressures at the cells are different. Even conceding such to be the case, it is difficult to visualize how decompression can exert a differential effect on the local circulation so as to bring about the observed results. It is clear that further investigation will be necessary before any final answer can be given.

SUMMARY

The oxygen consumption during and after activity was measured in six groups of subjects at various altitudes. Each of the groups performed a different exercise, four being modifications of the standard step-up and two, arm exercises. In all series except one the metabolic rate increased during the activity faster at an altitude of 30,000 feet than at sea level. The ratio of oxygen consumed during recovery to that consumed during activity was greater at altitude as was the length of time necessary for restoration of the resting level. These effects were shown in the arm series to become increasingly evident as the individual is decompressed from sea level to respectively 10,000, 18,000, 30,000 and 33,750 feet. When the exercise is relatively isotonic and localized, decompression increases the total oxygen consumption due to the exercise. When the muscular contraction is isometric and localized, decompression decreases the total consumption.

It is concluded that the basic disturbance in the aerobic cell oxidation system described in previous reports manifests itself during muscular work by increasing the rate at which an oxygen debt is incurred and by retarding the rate of recovery, as well as by altering the total excess metabolism.

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THE EFFECT OF VARIED THIAMINE INTAKE ON THE GROWTH OF RATS IN TROPICAL ENVIRONMENT

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Opinion is still divided as to whether vitamin requirements vary with climate. It is important to establish these facts, because, if physiological reactions and nutritional requirements are different under varied climatic conditions, diets and therapeutic measures may have to be modified accordingly. A number of investigators have undertaken experiments dealing with vitamin requirements under conditions of high temperature and humidity, but the interpretation of the results has not yet resulted in agreement (1–7).

The following investigation is a study of varied intake of certain B vitamins, with special emphasis on thiamine, in relation to growth and food consumption of rats as influenced by tropical environment.

METHODS USED IN LIVER ASSAYS. The procedure used for the determination of thiamine was a modification of the thiochrome method of Hennessy and Cerecedo (8). Riboflavin was determined by the method of Snell and Strong (9), and pantothenic acid by the method of Pennington, Snell and Williams (10), and by the technique of Silber and Mushett (11). Nicotinic acid was determined by the method of Snell and Wright (12).

EXPERIMENTAL PROCEDURE. The conditions used in this study were 90°F. and 70 per cent humidity for the experiments in tropical environment, contrasted with temperate conditions of 72°F. and 50 per cent humidity. In the description of the following experiments the terms "tropical" and "temperate" refer specifically to these conditions.

The rats were maintained on an adequate stock diet before receiving the special diet used in these experiments. The experimental diet consisted of casein (vitamin-free, Harris) 18 per cent; dextrose 68 per cent; salt mix U.S.P. no. 1, 4 per cent; hydrogenated vegetable oil (Crisco) 8 per cent; cod liver oil 2 per cent. The vitamins added were 0.2 mgm. thiamine hydrochloride, 0.2 mgm. riboflavin, 0.3 mgm. pyridoxine, 0.5 mgm. nicotinic acid, 0.05 gram choline chloride, 0.05 gram inositol, and 0.375 gram whole dried liver per 100 grams of diet. The vitamins and liver were either incorporated in the diet or administered separately when it seemed desirable to vary the intake in relation to the other dietary constituents. The procedure used is specified in each case.

The plan of each experiment was similar. For each group in the tropical environment, there were two in the temperate environment—one fed the same diet ad libitum, another restricted to the identical caloric and vitamin intake of the tropical group.

The standard error of the mean is stated for numerical values. The data have been statistically evaluated using the F and t tests described by Snedecor (13).

Graphs shown represent per cent weight, taking the initial weight as 100.

Experiment 1. The object of this experiment was to ascertain the influence of tropical environment on rats with varied intake of certain of the B vitamins.

The test animals were 48 male albino rats with an average weight of 344 ± 7.2 grams.

The vitamins were incorporated in the diet. Two groups received an additional supplement by oral dosing as indicated in table 1. Food consumption was measured daily and body weights recorded every other day.

The grouping of the animals, the daily average food consumption found, and the thiamine intake are shown in table 1.

After the rats had been in the tropics 81 days a stable weight response was established. The weight changes over this period are shown in figure 1. With the vitamins incorporated in the diet, the weight of the tropical rats (group I)

TABLE 1

Experiment I. Average food consumption and vitamin intake computed over an 81-day test period

GROUP	NO. OF RATS	ENVIRONMENT	FOOD	AVERAGE GRAMS FOOD DAILY	AVERAGE MICROGRAMS THIAMINE DAILY
I	12	Tropical	Ad libitum	10.0	20.0
II	12	Temperate	Ad libitum	19.7	39.4
III	12	Temperate	Restricted to intake of I	10.0	20.0
IV	6	Tropical	Ad libitum	11.0	22 42*
V	6	Temperate	Restricted to intake of IV	11.0	22 42*

* After 26 days of test these animals were given additional vitamins by oral dosing to approximate the intake of group II.

was 25 per cent inferior to the temperate rats feeding ad libitum (group II), and 25 per cent superior to the temperate restricted rats (group III).

The curves of the two tropical groups (groups I and IV) showed almost no divergence, while the two temperate restricted curves (groups III and V) varied significantly. The daily food consumption was higher in the groups receiving the greater vitamin allotment (see table 1) which resulted in a relatively greater increment in weight increase in temperate than in tropical rats.

To determine whether the greater weight of group V was due to the slightly higher caloric intake or the difference in thiamine intake, two temperate groups of 6 rats each (average weight 267 grams ± 5.97) were subsequently put on test and restricted to 10 grams of diet daily. The vitamin intake corresponded to the previous levels of restricted groups. Over a period of 46 days the weight curves differed by less than 3 per cent, demonstrating that the increased vitamin intake did not enhance weight under these conditions.

To assay the livers for certain of the B vitamins, 5 rats in each of the original groups were sacrificed after an 81-day test period. The livers were immediately

frozen after removal. The ratio of liver weight to body weight was not significantly different among groups. The results of these assays are shown in table 2.

The concentration in the livers of riboflavin, pantothenic acid and nicotinic acid showed no significant differences among any of the groups. However, thiamine did show differences among all but 3 groups as is indicated in table 2.

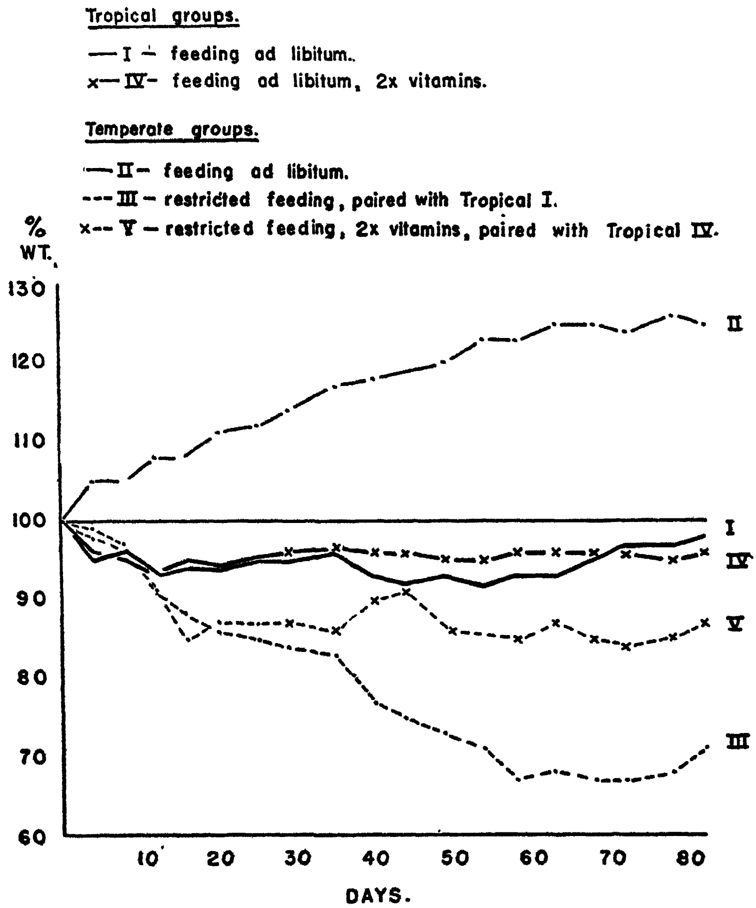

Fig. 1. Experiment I. Curves represent percentage of body weight taking the initial weight as 100. Groups correspond with those in table 1.

When the thiamine calorie ratio was the same, there was no significant difference in the liver thiamine of temperate rats, whether feeding ad libitum or on restricted ration, or between tropical and temperate rats feeding ad libitum, although the actual caloric intake was 50 per cent less in the tropics. Increasing thiamine in relation to caloric intake resulted in a proportionate increase of liver thiamine compared to corresponding groups receiving less thiamine in both tropical and temperate environment.

Experiment 2. A second experiment was carried out in which the relative

growth of rats receiving daily intakes of 5, 10 and 30 micrograms of thiamine in both tropical and temperate conditions was determined.

The same basal diet was used as that previously described, except that the dried liver was increased to 75 mgm. per rat per day and was given with the vitamins in caster cups. Therefore, in addition to the supplement every rat was receiving 0.2 microgram of thiamine daily from this liver. By feeding the vitamins in this way, the same vitamin intake in corresponding tropical and temperate groups was assured and the influence of varying levels of thiamine per se could be observed. All vitamin levels, with the exception of thiamine, were the same as in the foregoing experiment.

TABLE 2

Experiment I. Results of liver assays for thiamine, riboflavin, pantothenic acid, and nicotinic acid after an 81—day test period

The standard error of the mean is shown for the figures below. All thiamine values are statistically significant except those between groups I and II, between groups II and III, and between groups III and IV.

GROUP	ENVIRONMENT AND FEEDING	WT. 2 DAYS BEFORE SACRIFICE	WT. AT TIME OF SACRIFICE*	WT. OF WHOLE FRESH LIVER	LIVER WT. PER 100 GM. BODY WT.	MICROGRAMS PER GRAM FROZEN LIVER			
						Thiamine	Riboflavin	Panto-thenic acid	Nicotinic acid
		gm.	*gm.*	*gm.*					
I	Tropical ad libitum	328 ±8	316	8.09 ±1.47	2.56 ±0.23	2.28 ±0.16	43 ±4.32	77 ±8.46	135 ±11.3
II	Temperate ad libitum	448 ±42	434	11.50 ±1.36	2.56 ±0.96	3.53 ±0.36	45 ±6.45	72 ±13.15	123 ±9.52
III	Temperate restricted to I....	254 ±26	239	5.36 ±1.83	2.67 ±0.70	4.78 ±0.22	41 ±10.32	79 ±9.88	128 ±21.8
IV	Tropical ad libitum (additional vitamins).....	343 ±9	331	6.38 ±1.5	2.33 ±0.97	5.05 ±0.03	45 ±4.56	81 ±6.36	129 ±7.63
V	Temperate restricted to IV (additional vitamins)..... .	307 ±11	291	6.41 ±1.5	2.56 ±0.26	7.69 ±0.02	48 ±8.27	79 ±6.21	136 ±8.40

* Deprived of food 24 hours before sacrifice.

The 90 rats used were male and female albinos bred at the Merck Institute and born within 4 days of each other. The nine groups were composed of 5 males and 5 females each and were arranged as shown in table 3.

After 85 days, when the tropical groups appeared to have reached a fairly stable plateau, all rats receiving 30 and 10 micrograms thiamine per day were reduced to 2 micrograms daily.

Consistent with the previous experiment, the food consumption of the tropical rats feeding ad libitum dropped between 30 per cent and 50 per cent below that of the corresponding temperate groups. Since these rats were younger, and growth was normally more rapid, the weight of all groups showed a greater percentage increase than in the preceding experiment (expt. 1). Although the actual weight of the females was less than that of the males, their response to

the conditions of the experiment was relatively similar. The same basic differences were apparent as in the previous experiment.

The weight response of the male groups is shown in figure 2.

Oxygen consumption determinations were made on all the rats in this experiment using the Richards and Collison technique (14). A slight decrease in oxygen uptake in the tropics was observed, but the question has arisen whether or not the testing chamber in this apparatus at the higher temperature became saturated with moisture, making heat loss difficult for the animal and subjecting him to conditions different from the experimental tropical environment.

TABLE 3

Experiment II. Organization of groups

Curves for the males are shown in figure 2. The standard error of the mean is given for the weights.

GROUP	ENVIRONMENT	NUMBER OF RATS				MICROGRAMS THIAMINE PER RAT PER DAY	FEEDING
		Male	Av. wt. at start	Fem.	Av. wt. at start		
XII A	Tropical	5	190 ±11.2	5	170 ±6.7	30	Ad libitum
XIII A	Tropical	5	216 ±10.9	5	157 ±6.6	10	Ad libitum
XIV A	Tropical	5	214 ± 9.46	5	172 ±7.06	5	Ad libitum
XII B	Temperate	5	199 ±19.42	5	167 ±5.6	30	Ad libitum
XIII B	Temperate	5	218 ±10.6	5	158 ±5.16	10	Ad libitum
XIV B	Temperate	5	213 ±14.4	5	166 ±3.62	5	Ad libitum
XII C	Temperate	5	209 ±12.8	5	177 ±8.27	30	Restricted to XII A
XIII C	Temperate	5	211 ±12.8	5	152 ±9.0	10	Restricted to XIII A
XIV C	Temperate	5	212 ±16.4	5	165 ±8.6	5	Restricted to XIV A

Experiment 3. The purpose of this experiment was to extend the range of thiamine levels from a deficient intake to a massive intake of 300 micrograms of thiamine per day. The work was organized in the same manner as heretofore. Nine groups of 7 male albino rats each were used having an average weight of 242 grams. The basic diet was the same as in the preceding experiments. The liver (75 mgm.) and the vitamin supplement were fed in caster cups. Therefore, in addition to the vitamin supplement each rat was receiving 0.2 microgram thiamine per day from this liver. The vitamin levels remained the same as before with the exception of thiamine. In order to compare the uniformity of response of the groups in each category, all the rats received 30 micrograms of thiamine per day for the first 23 days. Thereafter, three levels of daily thiamine intake—300 micrograms, 30 micrograms and a deficient intake were started as shown in table 4. The results of this experiment are graphically presented in figure 3.

Food consumption of the tropical groups feeding ad libitum again dropped 30 per cent to 50 per cent below that of the temperate groups feeding ad libitum. Rats exhibited no better growth when receiving 300 micrograms thiamine per day than when receiving 30 micrograms in either temperate or tropical environment, since statistically no significant difference existed between temperate and tropical groups at these levels of thiamine intake.

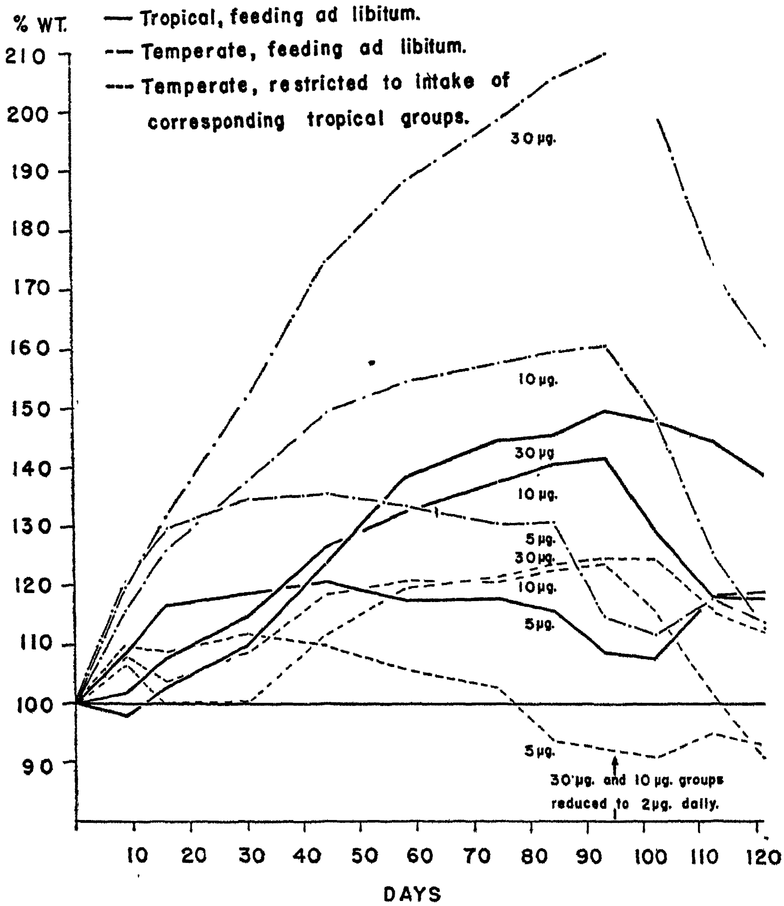

Fig. 2. Experiment II. Curves represent percentage of body weight taking the initial weight as 100. Daily thiamine intake as indicated. Break in uppermost curve due to the death of one rat.

Striking differences existed between temperate and tropical rats on the thiamine deficient diet in regard to weight loss, incidence of polyneuritis, and the length of time before its manifestation as shown in figure 3.

In the temperate group feeding ad libitum, 5 rats became polyneuritic; in the temperate group on restricted ration, 2 rats; and, in the tropical group, 1 rat. All others died without developing polyneuritic symptoms.

To compare weight loss, a time was chosen when at least 4 rats remained in each deficient group to constitute a representative average. This was 34 days after initiating the thiamine deficient diet. The temperate group feeding ad libitum had declined 44 per cent; the temperate restricted group, 23 per cent; the tropical group feeding ad libitum, 16 per cent.

DISCUSSION. When introduced into a tropical environment (90°F. and 70 per cent humidity) the weight of rats dropped abruptly, but after 10 to 14 days the initial weight was regained and in the case of growing rats a more stable, gradual response was attained.

The food consumption of rats in tropical conditions (90°F., 70 per cent humidity) decreased 30 per cent to 50 per cent compared to that of rats in temperate conditions (72°F., 50 per cent humidity) and receiving the same amounts of thiamine. This anorexia was not due to a thiamine deficiency because it was

TABLE 4

Experiment III. Organization of groups and initial weights

GROUP	ENVIRONMENT	FEEDING	NUMBER OF RATS	AV. WT. AT START	MICROGRAMS THIAMINE PER DAY
XX A	Tropical	Ad libitum	7	241 ±6.76	300
XXI A	Tropical	Ad libitum	7	241 ±8.86	30
XXII A	Tropical	Ad libitum	7	243 ±7.59	0
XX B	Temperate	Ad libitum	7	241 ±9.04	300
XXI B	Temperate	Ad libitum	7	242 ±6.79	30
XXII B	Temperate	Ad libitum	7	241 ±3.47	0
XX C	Temperate	Restricted to XX A	7	243 ±6.54	300
XXI C	Temperate	Restricted to XXI A	7	241 ±7.88	30
XXII C	Temperate	Restricted to XXII A	7	242 ±6.65	0

manifested at daily intakes as high as 30 and 300 micrograms of thiamine, and was evidenced immediately upon exposure to tropical conditions.

With this decrease in food consumption, the weight of tropical rats was 15 per cent to 25 per cent superior to that of temperate rats restricted to identical dietary consumption, and was 15 per cent to 25 per cent below the response of temperate rats feeding ad libitum in all but one experiment, where tropical weight response showed no significant difference from the temperate. When the vitamin supplement was fed in caster cups, the reduction in tropical caloric intake proportionately increased the thiamine-calorie ratio. This increased ratio did not enhance their weight, since they reached a ceiling in growth response at the same, or a lower level of thiamine intake than temperate groups.

When tropical and temperate rats receiving adequate thiamine per day (30 or 300 micrograms) were reduced to 2 micrograms daily, or to an even more deficient diet, the weight loss gradient was far more gradual in the tropics than in temperate conditions. This fact is substantial evidence that tropical thiamine requirements for growth are not greater than temperate. Due to re-

duced food consumption these tropical rats were receiving more thiamine per calorie than their temperate controls. If thiamine metabolism were altered in such a way as to increase the requirement for growth in the tropics, such a deficit should have been demonstrated by weight loss equal to or greater than the temperate loss.

Mills (4) has reported that thiamine requirements for weanlings are doubled in tropical conditions, but his experimental data on low thiamine intake seem

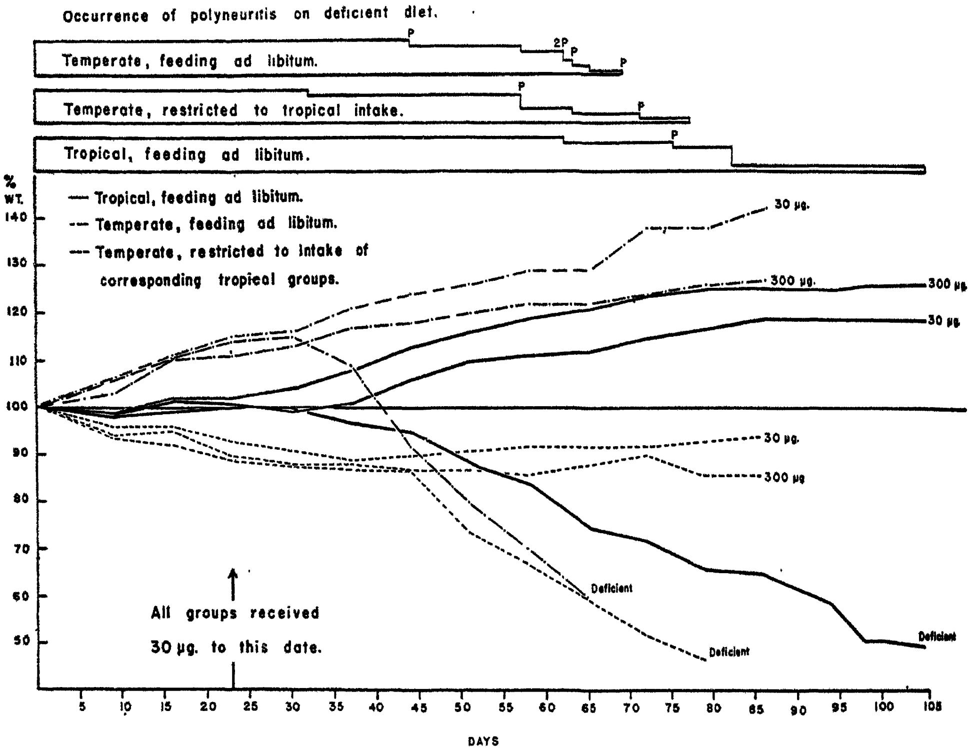

Fig. 3. Experiment III. Curves represent percentage of body weight taking the initial weight as 100. Daily thiamine intake as indicated. No significant difference exists statistically among any of the four curves above the 100 base line. Blocks show occurrence of polyneuritis indicated by letter *P;* drops without a letter indicate death without polyneuritic symptoms.

to confirm our interpretation. He shows that when using a diet containing 0.2 mgm. thiamine per kgm. food mixture, his cold room (65°F.) rats lost 43 grams in weight, while the hot room (90°–91°F., 60 per cent R.H.) rats lost only 18 grams during the last 4 weeks of test. The thiamine-calorie ratio was the same for both groups. Nevertheless the hot room rats did not show a more rapid decline in weight than those in the cold room.

Assays of liver for certain of the B-vitamins showed that when the thiamine calorie ratio was the same, the liver thiamine concentrations were also compar-

able in both tropical and temperate conditions in rats feeding ad libitum. There was no significant difference among tropical and temperate groups in concentration of riboflavin, pantothenic acid, or nicotinic acid.

The incidence of polyneuritis was greater and appeared earlier in temperate than in tropical conditions when rats were fed a thiamine deficient diet.

In physiological studies on the effect of tropical conditions on the organism, it is necessary to distinguish between accomplished acclimatization and the early transitional period, if animals are abruptly introduced into tropical conditions.

Since changes in environmental conditions produce quantitative differences in certain physiological responses, it seems obvious that specifications of temperature and humidity are as important data in reports of nutritional and pharmacological studies as are the quantities of the nutrients and drugs administered. Under varied climatic conditions diet and therapeutic measures may have to be modified to obtain the most effective results.

SUMMARY

Thiamine requirements for the growth of rats in a tropical environment (90°F and 70 per cent relative humidity) are not greater and may be less than in temperate conditions (72°F. and 50 per cent relative humidity).

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INTERRELATIONS OF ADRENAL AND SEX GLANDS IN PARABIOTIC RATS[1]

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Ovarian secretion or the injection of estrogenic hormones induces some hypertrophy of the adrenal cortex, while the testicular secretion or the injection of androgenic hormones on the contrary produces a slight atrophy of the adrenal cortex.

The weight of the adrenal glands is definitely correlated with sex. In rats the adrenals are larger in females, absolutely, and in relation to body weight at all ages (4). Castration causes atrophy of the adrenal cortex in the female and hypertrophy in the male rat (8, 9). The stimulating action of the ovaries and of the estrogenic hormones is not observed in hypophysectomized rats, intact or castrate.

In this paper the relations between the adrenals and the gonads are studied by means of parabiosis, which has the advantage of being a physiological and not a pharmacological method.

Material and methods. Adult white rats (from a strain with twenty years of inbreeding) weighing between 160 and 170 grams were used. The parabiotic union between both animals was always ample: a longitudinal incision that went from the root of the forelimb to the hindlimb; both rats were joined not only by the skin but also by caelioanastomosis. Castration was performed by dorsal route. The sexual hormones dissolved in oil were injected subcutaneously.

Results. *Adult female rats united with castrate parabionts (female).* The stimulating action of the ovary and of estrogens on the adrenal cortex is observed by experiments in adult female rats united with castrate parabionts (8). The results are summarized in table 1.

Female rats, castrated 15 days before, were united with normal animals and both were killed on the 15th day of their parabiotic life. The adrenals of the intact animals showed hypertrophy (71.3 mgm.), while the average adrenal weight of intact females was 54.6 mgm. after 15 days of parabiotic life with an intact partner. Parabiosis between two castrate rats gave an average adrenal weight of 47.5 mgm. (table 1).

The adrenal increase in weight may be attributed to the stimulation and resulting hypertrophy of the ovaries in the intact rat brought about by the excess of circulating gonadotrophins secreted by the hypophysis of the spayed parabiont. The ovarian stimulation is shown by the marked increase in weight of the intact rat's ovaries (102.8 mgm. average weight). These glands had

[1] The experiments were made under the direction of Prof. B. A. Houssay.

many large follicles and sometimes corpora lutea. Permanent estrus was found in these rats (in some cases in both animals) and also an increase in weight of the uterus (4, 5 to 10, 12).

Histological examination of the adrenal cortex showed hyperplasia and hypertrophy of the cells of the reticular zone and internal part of the fascicular zone.

Adult female rats united with castrate parabiont treated with estrogens. In another series intact rats were united with rats castrated 15 days previously; the castrates were injected from the first day of parabiosis, in one group with 2 I.U. and in another group with 5 I.U., of estradiol benzoate daily during 15 days, with the object of suppressing hypersecretion of gonadotrophins in the spayed rat, thus preventing hypertrophy and hyperfunction of the ovaries in the intact rat, and still permitting the study of the modifications of the adrenals.

TABLE 1

Parabiosis of female rats; 15 days of parabiotic life

(Standard deviation between parentheses)

LOT NO.	NO. OF PAIRS	AVERAGE BODY WEIGHT	ADRENALS (A)	OVARIES (A)	ADRENALS (B)	OVARIES (B)
Intact rat (A) with rat castrated 15 days before (B)						
		grams	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	10	162.5	71.3 (±2.37)	102.8	52 (±1.66)	
Intact rat (A) with rat castrated 15 days before and receiving 2 I.U. of estradiol benzoate daily (B)						
2	3	161.6	58.6 (±3.09)	63.3	59.3 (±2.15)	
Intact rat (A) with rat castrated 15 days before and receiving 5 I.U. of estradiol benzoate daily (B)						
3	3	168.3	55.3 (±2.24)	44	59 (±1.25)	
Intact rat (A) with intact rat (B)						
4	8	166.8	54.3 (±0.92)	51.1	55.2 (±0.42)	62.5
Rat castrated 15 days before (A) with rat castrated 15 days before (B)						
5	8	159.3	48.2 (±1.05)		47.2 (±0.80)	

The results obtained showed that both these doses of estrogens efficiently prevented hypersecretion of gonadotrophins, and so prevented hypertrophy of adrenals in the intact rat. The respective weights of both organs can be seen in table 1. Histologically the adrenals gave a completely normal picture.

The same effect was also obtained by successful grafts of ovaries in the ear or kidney of the castrate rat (8).

Adult male rats united with castrate parabionts (male or female). While the ovarian hormones stimulate the development of the adrenal cortex in female rats, the testicular hormones on the contrary have a depressing action on the

TABLE 2

Parabiosis of male rats; 15 days of parabiotic life

(Standard deviation between parentheses)

LOT NO.	NO. OF PAIRS	AVERAGE BODY WEIGHT	ADRENALS (A)	SEMINAL VESICLES (A)	ADRENALS (B)	SEMINAL VESICLES (B)
Intact rat (A) with rat castrated 30 days before (male) (B)						
		grams	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	5	175	31 (±1.20)	1.120	45.2 (±2.12)	30
Intact rat (A) with rat castrated 30 days before (female) (B)						
2	4	172.5	31 (±1.33)	1.100	46.8 (±0.80)	
Intact rat (A) with intact rat (B)						
3	4	166.2	40.2 (±1.14)	317.5	39.5 (±1.70)	321
Castrated rat (A) with castrated rat (B)						
4	4	175	49.5 (±0.94)	64.2	51 (±2.07)	54.5
Intact rat (A) with rat recently castrated and receiving 2.5 mgm. of testosterone propionate daily (B)						
5	3	173.3	36 (±0.77)	600	35.3 (±0.95)	1,966.6
Intact rat (A) with rat castrated 30 days before and receiving 2.5 mgm. testosterone propionate daily (B)						
6	4	177.5	37 (±1.70)	550	49.2 (±0.92)	1,375
Intact rat (A) with rat castrated 30 days before, receiving 5 mgm. testosterone propionate daily (B)						
7	4	171.2	34.5 (±0.19)	937.5	33.2 (±0.34)	1,912.5

adrenal cortex of adult male rats (1, 2, 3, 11). This action of the testes was demonstrated by experiments in adult male rats united in parabiosis (table 2).

Adult male rats castrated 15 days before were united with intact male rats and both were killed on the 15th day of their parabiotic life. An evident decrease in the adrenal weight of the intact partner was observed: their adrenals weighed 31 mgm. as compared with 39.8 mgm. in control intact male rats living in para-

biosis. This effect is due to hypersecretion of testicular hormones in the intact animal brought about by hypersecretion of gonadotrophins in the castrated partner. Hypersecretion of male hormones by the testicles of the intact parabiotic rat was made evident by the observed marked hypertrophy of the seminal vesicles (table 2). Histologically, the atrophied adrenals of the intact rat showed a decrease in size of the cells of the reticular and internal part of the fascicular zones.

The adrenal weight of male castrate rats united with castrate parabionts was greater (average 50 mgm.) than that of parabiotic intact male rats (average 39.8). This increase is due to the effects of castration. Adrenal hypertrophy of the same degree is seen in individual castrates (5, 9).

In another series the effect of castration on the hypophysis was corrected by the injection of varying doses of testosterone propionate, thus preventing testicular hyperfunction in the intact companion. *The daily injection of 2.5 mgm. of testosterone propionate to recently castrated rats united with intact parabionts* prevented adrenal hypertrophy in the castrate partner (35.3 mgm. compared to 50 mgm. in the controls), and prevented atrophy of the adrenals of the intact animal (36 mgm. compared to 31 mgm. in the controls) (table 2).

When a rat castrated 30 days before was united by parabiosis with an intact rat, it became necessary to inject 5 mgm. of testosterone propionate into the castrate animal, in order to prevent the adrenal hypertrophy following castration; this dose not only prevented adrenal hypertrophy in the castrate animal, but even caused a slight adrenal atrophy in the intact partner (average weight 33.2 mgm.) demonstrating that some of the testosterone injected in the castrate passed into its intact partner; this fact was confirmed by the hypertrophy of seminal vesicles observed in the intact rat (table 2). The adrenals of the castrate rats also showed the effect of testosterone since their weight fell below that of controls (table 2).

Discussion. Many authors have used parabiosis of castrate with intact rats of both sexes in order to demonstrate the increase of gonad-stimulating substances secreted by the hypophysis of castrate animals. It was found in this way that the sex glands of the intact animal were strongly stimulated, as shown in the female by the marked hypertrophy of the ovaries and uterus and by the presence of prolonged estrous cycles, and in the male by the considerable hypertrophy of seminal vesicles.

Parabiosis of a castrate rat with an intact female causes an extraordinary hypertrophy of the ovaries with a first phase of follicular hypertrophy and a second phase of evident luteinization When these animals were killed between the 10th and the 15th day of their parabiotic life, hypertrophy of ovaries in the first phase was evident—i.e., there was follicular ripening, although a moderate quantity of corpora lutea were already present. In this case the adrenals showed an evident hypertrophy due especially to hyperplasia and hypertrophy of the cells of the reticular zone with vasodilatation. This adrenal hypertrophy can be explained by the excess of estrogens circulating in the intact animal, whose hypertrophied ovaries are responding to the excess of gonadotrophins, produced by the hypophysis of the castrate animal.

In some cases the excess of estrogens in the blood of the intact animal invaded the circulation of the castrate, a fact already demonstrated by the presence of an estrous phase in the vaginal cycle of the latter. When this happened, the ovaries and adrenals of the intact parabiont showed a smaller increase in weight, and at times the adrenals of the castrate partner also increased in weight and there was no atrophy of the reticular zone.

The adrenal hypertrophy in the intact rat united with a castrate parabiont is probably due to the following mechanism: the excess of gonadotrophins produced by the castrate rat's hypophysis brings about the hyperfunction of the intact rat's ovaries which secrete an excess of estrogenic hormone; these hormones produce the stimulation of the adrenotrophin secretion of the hypophysis in the intact rat, which in its turn provokes the adrenal cortex hypertrophy in the same animal. The exaggerated secretion of hypophyseal gonadotrophins induced by castration can be prevented by the injection of estradiol benzoate (from 2 to 5 I.U.) or a successful ovarian grafting (8) in the spayed rat.

Moreover, in another paper (8) it was demonstrated that when a female intact rat was united in parabiosis with a castrated hypophysectomized rat, there were no changes in the adrenal cortex of the intact partner; this fact shows the necessary presence of the hypophysis adrenotrophic secretion for the production of adrenal cortical hypertrophy in the female intact rat united in parabiosis with a castrate male or female rat.

In the second series (parabiosis of a male or female castrate with an intact male) testicular hyperfunction was evident from the marked hypertrophy of the intact animal's seminal vesicles observed at the end of 15 days. The same animal had a decrease in weight and size of the adrenals, due to atrophy of the cells of the reticular zone and of the internal part of the fascicular zone. This change may be explained as due to the increase of circulating male hormone in the intact animal as a result of the increased secretion of its testicles under the influence of the gonadotrophins secreted by the castrate's hypophysis. Moreover, this excess of circulating male hormone in the intact partner probably decreased the hypophysis adrenotropic secretion in the same animal.

In order to prevent the increase in gonadotrophin production 2.5 mgm. of testosterone propionate were administered to the castrate, with the result that the seminal vesicle and adrenals of the intact parabiont remained normal, while the adrenals of the castrate partner showed normal weight and histology.

To produce the same effect a larger dose of male hormone is necessary if the castration is performed 30 days before parabiosis. In some cases, when high doses of testosterone propionate (5 mgm.) had been used, not only restitution to normal of the castrate's adrenals, but also a partial atrophy of the intact rat's adrenals, can be demonstrated. This is probably due to the fact that some of the hormone injected in the castrate passed into the intact partner, as demonstrated by an increase in weight of seminal vesicles in the latter.

SUMMARY AND CONCLUSIONS

The relations between the adrenals and the gonads were studied in rats by means of parabiosis. Adult rats, male or female, were united with castrate

parabionts and a study was made of the effect of gonad stimulation on the adrenal cortex.

1. Ovarian stimulation obtained by parabiosis causes hypertrophy of the adrenal cortex. On the other hand, stimulation of the testes by the same method causes atrophy of the adrenal cortex. The mechanism of these adrenal changes is discussed.

2. The experimental results obtained indicate that the ovarian secretion has a stimulating effect on the development and maintenance of the adrenal cortex, especially on the inner zones (reticular and fascicular). The testicular secretion has an inhibiting action on the same zones (reticular and fascicular).

3. This means that the adrenal cortical function is in great part regulated by gonadal influences and is therefore of a sexual character.

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EFFECT OF ISOLATED POSTERIOR PITUITARY PRINCIPLES ON SURVIVAL OF THE PRIMITIVE RESPIRATORY CENTER IN THE DECAPITATED RAT HEAD[1]

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It has recently been demonstrated (1) that anterior or posterior pituitary extracts markedly prolong the period of gasping of the ischemic decapitated rat head. The factors responsible for this result are most likely the diabetogenic hormone of the anterior lobe and one or both of the known posterior lobe hormones. It is not improbable that commercial anterior lobe preparations may contain some impurities (of the posterior lobe). Any reaction causing hyperglycemia should prolong the life of the respiratory center(s) insofar as the carbohydrate becomes available (1, 2). It was reported (3) as early as 1908 that extracts of the posterior pituitary injected into rabbits produce glycosuria and hyperglycemia.

The literature shows diversity of opinion of the hyperglycemic action of vasopressin and/or oxytocin. Some have reported the pressor principle, others the oxytocic one solely as the hyperglycemic agent. It is possible that impurities (of oxytocin, vasopressin, or anterior lobe) may have influenced their results. It appears more likely to us that the pressor principle is the more powerful (if not the only one) in producing hyperglycemia. (For an extensive review see Van Dyke, 4.)

White rats of the Wistar strain between 40 and 50 grams (of approximately the same age) were injected intraperitoneally with 5 units of vasopressin[3] or 2.5 units of oxytocin[3] and decapitated 10 minutes later by a single stroke of a razor blade. The subsequent gasps were recorded manually on an electrically driven kymograph which also served as a chronometer. As soon as the head was severed from the body, blood was drained from the latter for blood sugar determination by the Folin and Wu micro-method. It was observed that much less blood escaped from the animals receiving vasopressin than from those receiving oxytocin or from the controls. Two possibilities seemed apparent: *a*, hemoconcentration and/or *b*, vasoconstriction. In order to ascertain whether or not hemoconcentration occurred, erythrocyte counts were made from both groups of rats before and after injection, the results showing no significant variation in red cell number indicating that the pressor action caused enough hemo-

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[3] Pitressin and pitocin supplied by Oliver Kamm, Scientific Director, Parke, Davis & Co., Detroit, Mich.

stasis to reduce the rate of hemorrhage. Blood sugar determinations showed no significant change following oxytocin but a significant rise after vasopressin (see table 1). It is thus apparent that of the two known hormones of the posterior pituitary the pressor principle alone in our experiments was responsible for both prolonging the survival time and increasing the blood sugar level. It has recently been reported (5) that the aromatic sympathomimetic amines causing hyperglycemia also prolong the survival of the respiratory center. That a rough but significant correlation between glycemic level and anoxic survival exists has been found (6). Other factors may also be important in anoxic survival, such as changes in the sensitivity of the respiratory center and autonomic excitation. However, the decapitated head is immediately devoid of any circulatory changes. Our results show no significant glycemic changes following oxytocin thus disagreeing with reports mentioned previously. Possibly the differences reported have been due in large part to impurities present or to the manner in which the substances were administered to the animal. In all cases

TABLE 1

Effects of vasopressin and oxytocin on total survival time, blood sugar, with percentage change from normal in isolated rat heads. The data show a significant change in hyperglycemia following vasopressin but an insignificant change following oxytocin

	NO. RATS USED	TOTAL SURVIVAL TIME		CHANGE FROM NORMAL	NO. RATS USED	BLOOD SUGAR		% CHANGE FROM NORMAL	t_{1-i}	P
		Mean	S.D.			Mean	S.D.			
		sec.	*sec.*			*mg. %*	*mg. %*			
Controls.........	23	38.2	4.13		21	107.4	7.85			
Vasopressin.......	32	63.8	11.9	+67.0	20	141.1	10.5	+31.4	8.726	<0.01
Oxytocin.........	28	39.9	7.4	+4.5	18	108.1	2.0	+0.65	0.2609	0.79

of the present study the hormones were injected intraperitoneally which may have resulted in inactivation of the oxytocin. It has been stated (7) that the blood sugar peak in cats is reached only 3 to 10 minutes after injection so the time interval we have allowed should be ample for a smaller animal such as the rat.

SUMMARY

Vasopressin ("pitressin") increases the total survival time of the primitive respiratory center in the isolated rat head. This is concurrent with a mild hyperglycemia. No hemoconcentration occurs although bleeding is greatly reduced.

Oxytocin ("pitocin"), even in large doses, does not prolong total survival time of the primitive respiratory center in the isolated rat head. No significant glycemic change occurs.

Therefore it is concluded that prolongation of survival of the isolated respiratory center following injections of aqueous extract of the posterior pituitary is due to the presence of the pressor principle, vasopressin, alone.

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METABOLIC CHANGES IN SHOCK AFTER BURNS[1,2]

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The preceding papers in this series (1–4) have reported on various aspects of the chemical changes resulting from hemorrhagic shock in rats. The present paper extends certain of these studies to the subject of burn shock in rats.

METHODS AND MATERIALS. Male albino rats of the Sprague-Dawley strain, weighing in most groups from 200 to 250 grams, were used in these experiments. The animals were fed a diet of Purina dog chow and in all cases were fasted 18 to 24 hours before being studied. During the period of fasting, and always subsequent to the thermal trauma, the rats were kept in individual cages with adequate drinking water in a room at a constant temperature of 83°F. Hypophysectomy was performed on rats weighing from 140 to 180 grams and was done under ether anesthesia 3 days before testing. The early burn experiments were done under sodium pentobarbital (nembutal) anesthesia administered intraperitoneally in a dose of 4 mgm. per 100 grams body weight. All subsequent burns were done using ether anesthesia. In all cases the corresponding controls were similarly fasted and anesthetized.

Initial blood samples were obtained from the cut tail and were taken into clean centrifuge or hematocrit tubes containing a small amount of heparin.[3] The final specimens were obtained just before death by cardiac puncture with the thorax open. Pentothal sodium was used preliminary to obtaining the final samples, and control analysis showed no difference in the results if these specimens were obtained from the cut tail or heart. For the blood ammonia analyses, the terminal blood samples were drawn from the vena cava (exposed under sodium pentobarbital anesthesia) into syringes coated with ammonia-free potassium oxalate, measured in the syringes, and delivered immediately into previously prepared Conway microdiffusion units. All other samples were centrifuged immediately, and the plasma was used for analysis. Separate hematocrit readings were made with Wintrobe tubes. The adrenals were removed immediately after cardiac puncture, stripped of fat and fascia, weighed on a torsion

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Yale University.

[2] The experimental work reported in this paper was performed in the laboratory of the Department of Physiological Chemistry, Yale University School of Medicine, while one of the authors (H. N. H.) was on leave of absence from the Department of Surgery, Johns Hopkins University.

[3] We are indebted to Dr. B. J. Brent, of the Roche-Organon Company, Inc., of Nutley, New Jersey, for a generous supply of heparin.

balance, placed in 10 ml. volumetric flasks, and immediately triturated with 4 ml. of alcohol-acetone mixture, 1:1.

Plasma and liver amino nitrogen determinations were made by the colorimetric method of Frame, Russell, and Wilhelmi (5); cholesterol by the method of Sperry (6); and ammonia and amide nitrogen by methods described in another paper of this series (7), in which the Conway microdiffusion technique was used in conjunction with a colorimetric procedure (8). The rates of respiration of slices of liver were measured in the Warburg apparatus according to methods described in a previous paper of this series (4).

Thermal trauma was induced in all cases by scalding. The anesthetized animal was grasped with two long forceps, one at the base of the tail and one at the scruff of the neck, and was quickly immersed up to the neck, so that only the head was kept out of the water. The water was kept at a standard temperature, and the immersion was for a standard period of time. The rats were not shaved, but the water contained several drops of a wetting agent (Duponol), so that the fluid penetrated between the hairs to a high degree. The burns were of three grades: mild (70°C. for 5 sec.), moderate (72°C. for 7 sec.), and severe (80°C. for 7 sec.). Control animals were immersed for similar periods of time in water at room temperature also containing the wetting agent. Eighty burned rats and 23 control animals were used in this study.

Results. *Plasma amino nitrogen.* In a series of 57 rats, including 23 controls and 34 scalded animals, all those subjected to thermal trauma showed an increase in plasma amino nitrogen. This rise held true within the limit of 24 hours after the scalds, during which the animals were observed as shown in table 1. It is seen that in some instances, e.g., the 80° 7-second burns, the control amino nitrogen values were more than doubled, the increase being as much as 150 per cent. Most of these rats had only 1 blood sample taken, that being at the indicated time of observation, just before being killed.

In a similar group of animals 2 blood samples were taken, a control sample at the beginning of the experiment from the cut tail, and a terminal sample before death from the heart. In 2 control rats terminal specimens showed an average decrease of 2.4 mgm. ±0.1, as compared to the initial specimen. In 10 rats scalded at 80° for 7 seconds, the terminal specimen showed an average increase of 6.9 mgm. ± 0.6, or expressed in terms of the control rats, an increase of 9.1 mgm. These results are another way of expressing the extent and constancy of the increase as already shown in the previous paragraph.

Table 1 also shows that hypophysectomy did not prevent the rise in amino nitrogen, if the degree of the scald were taken into consideration.

Figure 1 shows the increase in plasma amino nitrogen in a series of 9 rats killed at varying intervals after scalds of constant intensity (80° 7 sec.).

Adrenal cholesterol. In a series of 13 control rats, killed at varying intervals after anesthesia, the total adrenal cholesterol in milligrams per cent of adrenal weight varied from 2.7 to 5.8, averaging 3.9. A series of 8 rats burned at 80° for 7 seconds under pentothal anesthesia, killed at the end of 6 hours, averaged 1.9 mgm. per cent. One rat similarly burned and killed at the end of 24 hours

had a total cholesterol value of 0.8 mgm. per cent. Seven rats burned at 72° for 7 seconds, also under pentothal anesthesia, and killed at the end of 24 hours, had adrenal total cholesterol values averaging 0.8 mgm. per cent. Two rats

TABLE 1

Plasma amino nitrogen changes in experimental scalds in the rat

EXPERIMENT	NUMBER OF ANIMALS	TYPE OF SCALD	HOURS AFTER SCALDING	PLASMA AMINO NITROGEN
				mgm. per cent
Control	10		6	6.4 ±0.3
Control	2		12	6.2 ±0.2
Control	4		24	6.2 ±0.3
Burn	8	70° 5″	6	8.3 ±0.4
Burn	2	70° 5″	24	7.8 ±0.7
Burn	6	72° 7″	24	8.5 ±0.4
Burn	12	80° 7″	6	15.0 ±0.4
Burn	1	80° 7″	24	14.9 ±0.0
Hypophysectomy, control	7		6	7.3 ±0.2
Hypophysectomy, burn	4	70° 5″	6	10.3 ±0.2
Mock hypophysectomy, burn	1	70° 5″	1	9.0 ±0.0

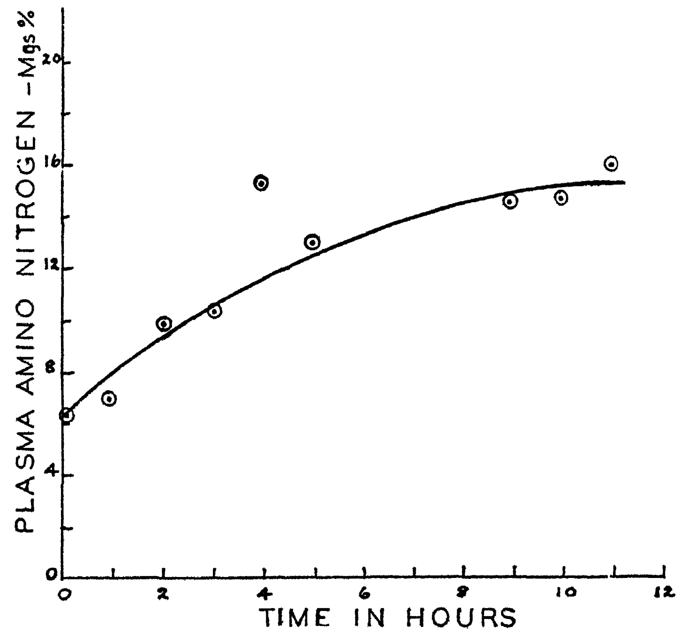

Fig. 1. The rise in plasma amino nitrogen following scalds at 80°F. for 7 seconds.

burned at 70° for 5 seconds under ether anesthesia, and killed at the end of 24 hours, had adrenal total cholesterol values averaging 1.4 mgm. per cent. One rat similarly burned and killed at the end of 48 hours had a value of 0.5 mgm. per cent. Five rats similarly burned and killed at the end of 72 hours had adrenal

cholesterol values averaging 2.3 mgm. per cent. These results demonstrate *a*, there is a marked fall in adrenal cholesterol following thermal trauma; *b*, the extent of the burn within the limits studied has much less effect on the degree of this fall than it does on the rise in plasma amino nitrogen as discussed in the previous section, and *c*, the lowest values seem to be attained in those rats killed at the end of 24 or 48 hours and are not so marked in those tested at the end of 72 hours. This latter point may be influenced by the survival of only the less severely burned rats for the longer interval.

Additional rats were burned under more constant conditions, all with ether anesthesia and all killed at the end of 6 hours. These, plus 6 of the above cited controls also done under ether, are analyzed in table 2. It is seen that the rise in hematocrit and in amino nitrogen in the 3 groups of burned rats is roughly similar, indicating an equal burn trauma insofar as these readings are

TABLE 2

Adrenal total cholesterol changes in experimental scalds in the rat

EXPERIMENT	INTRAPERITONEAL THERAPY	NO. OF RATS	ADRENAL TOTAL CHOLESTEROL MGM./100 MGM. ADRENAL WT.	AMINO NITROGEN	RISE IN HEMATOCRIT
				mgm. %	%
Control...........	None	6	3.6 ±0.3	6.1	0*
Burn 70° 5″........	None	8	1.0 ±0.1	8.3	9
Burn 70° 5″........	7.6 cc.† adrenal cortical extract	8	1.7 ±0.2	8.8	11
Burn 70° 5″	7.6 cc. alcohol saline	8	1.5 ±0.1	7.5	8

* Actually the control hematocrits fell an average of 4 per cent. For clarity of tabulation, this fall is used as a baseline of reference for the other figures given below in the same column.

† Divided into five approximately equal doses given at hourly intervals beginning with the first dose immediately after the burn.

concerned. The fall in adrenal cholesterol, however, was greater in the untreated burns than in those given alcohol-saline or adrenal cortical extract intraperitoneally. From the statistical standpoint the probability that this difference could be due to sampling of the results as shown in the table was in each instance 0.01 or less. These results are suggestive but not conclusive and tend to indicate the value of the therapeutic measures used.

One control rat had a very low adrenal cholesterol (0.5 mgm. per cent). It was noted that this animal's adrenals weighed 60 mgm. whereas in no other control experiment did the adrenal weight exceed 40 mgm. It was decided, therefore, that this rat was abnormal, and so its figures are not included in the averages. Two burned rats, nos. 6 and 75, also had adrenals weighing over 40 mgm., namely, 48 and 47 mgm. respectively, with accompanying cholesterol values of 0.8 and 0.3, the latter figure representing the lowest value found in any burned rat. These animals were also considered abnormal and are not included in the averages.

The adrenal cholesterol was tested in 2 rats which were found dead, one 6 hours and the other 72 hours after burning. The concentrations were 0.5 and 0.8 mgm. per cent of adrenal weight, respectively. These represent low values, but are not lower than those found in certain other burned rats whose adrenals were taken at the time of death. The lowest group of values was in a group of 7 rats burned at 72° for 7 seconds under pentothal anesthesia and killed at the end of 24 hours. Readings of the adrenal cholesterol (mgm. per cent of adrenal weight) varied from 0.6 to 1.0, averaging 0.8. An additional rat killed at the end of 48 hours showed an adrenal cholesterol value of 0.5.

In contrast to the marked decrease in adrenal cholesterol of normal rats when scalded is the behavior of this adrenal constituent in hypophysectomized rats submitted to the same degree of trauma. Thus, in a series of 8 rats submitted to hypophysectomy by the parapharyngeal approach 3 days before, scalds for 5 seconds at 70°F. did not evoke the usual changes in the adrenal total cholesterol. As seen in table 3, the value in 5 normal unburned animals was 3.7 ± 0.6 mgm. per cent. In either burned control animals or in burned animals previously subjected to a mock hypophysectomy, there was a marked fall to 1.0 ± 0.1 or 1.5 ± 0.2 respectively. On the other hand, 8 hypophysectomized animals which were burned to a similar degree showed no fall in the adrenal total cholesterol, the values averaging 5.7 ± 0.6 (the lowest value in this series was 3.8). The slightly higher values noted in hypophysectomized rats agree with the observations of Sayers et al. (10).

TABLE 3

*The effect of hypophysectomy in preventing the usual changes in the adrenal total cholesterol following experimental scalds in the rat**

PREPARATION	EXPERIMENT	NO. OF ANIMALS	RISE IN HEMATOCRIT	AV. BODY WT.	ADRENAL WT. AS MGM. % BODY WT.	ADRENAL TOTAL CHOLESTEROL MGM./100 MGM. ADRENAL WT.
			%	*grams*		
Control rats.	Control	5	0†	196	17	3.7 ±0.6
Control rats	Burn 70° 5″	8	11	231	15	1.0 ±0.1
Mock hypophysectomy...	Burn 70° 5″	3	16	171	22	1.5 ±0.2
Hypophysectomy.........	Burn 70° 5″	8	13	167	13	5.7 ±0.6

* All animals killed at the end of 6 hours.

† Actually the control hematocrits fell an average of 6 per cent. For clarity of tabulation, this fall is used as a baseline of reference for the other figures given below in the same column.

Blood and liver ammonia nitrogen, liver amide and amino nitrogen. As seen in table 4, in a series of 9 scalded rats subjected to analysis at varying intervals after trauma, there was an increase in the whole blood ammonia nitrogen. To some extent this rise became more marked over the period of 12 hours of observation. There was a slight, but probably insignificant rise in the liver ammonia nitrogen and no rise in the liver amide nitrogen. On the other hand, the liver amino nitrogen was definitely elevated in most instances. This rise seemed to

have no relation to the time after the burn as did the increase in blood ammonia nitrogen.

Oxygen uptake of liver slices. As seen in table 5, there is no early impairment of the oxygen uptake of liver slices from animals sacrificed at varying intervals after 80° 7 second scalds. Many of the values fell in the high normal range. This is in agreement with similar observations of Clark and Rossiter (9) on the respiration of liver slices from rats in shock after burns.

TABLE 4

Changes in blood and liver ammonia nitrogen and liver amide and amino nitrogen at varying intervals following an 80° 7″ scald in rats

RAT NO.	BODY WT.	TIME OF SACRIFICE	RISE IN HEMATOCRIT	BLOOD	LIVER		
				Ammonia N	Ammonia N	Amide N	Amino N
				Scalds, 80°7″			
	gm.	hours	%	mgm. %	mgm. %	mgm. %	mgm. %
1	232	1	+2	0.25	3.6	4.6	65
2	230	2	+10	0.18	3.0	5.9	68
3	226	3	+4	0.22	3.4	4.8	63
4	212	4	+6	0.19	3.6	7.0	73
5	236	5	+18	0.33	3.1	6.1	65
6	224	9		0.31	3.1	3.3	60
7	224	10		0.28	2.8	1.1	60
8	214	11		0.37	3.7	3.7	65
9	247	12		0.50	5.0	4.1	82
Average.....................			+8	0.29	3.5	4.5	67
				Controls			
10	230	13			2.5	6.9	58
11	222	13			2.4	6.8	54
Usual control range.				<0.1	2–4; av. 2.6	5–12	47–62; av. 54

Discussion. The increase in plasma amino nitrogen which occurs after burns in rats is as marked as that which has been observed after hemorrhage in rats (1), and greater than that noted by Hoar and Haist (11) in experimental tourniquet shock in dogs and in rats. In the present experiments the rise in plasma amino nitrogen may have been the result mainly of an increased rate of breakdown of protein due to the direct action of the thermal trauma. Whether some loss of liver function also occurred is not certain. No diminution in the rate of respiration of liver slices taken from these animals was observed, whereas in hemorrhagic shock there is a marked depression in the rate of respiration of the liver slices which can be correlated roughly with low rates of deamination and of urea formation. However, normal oxygen uptake rates are not necessarily indicative of complete normality of liver function, for Clark and Rossiter (9) found that liver slices from burned rabbits did not form glycogen although the slices respired normally. The high levels of free amino nitrogen in the

livers of the scalded rats, which appear too great to be accounted for solely by the increases in plasma amino nitrogen, may be the result of some alteration in liver function, as may also be the high blood ammonia levels. Hoar and Haist considered that the elevation of plasma amino nitrogen in tourniquet shock was due mainly to contribution of amino acids from the injured limbs, but they also obtained evidence of impaired liver function in their preparations. It may be noted that whatever the origin of the elevation in plasma amino nitrogen, it is not dependent on the presence of an intact hypophyseal-adrenocortical system.

Muus and Hardenbergh (12) studied the effects of burns on the metabolism of the liver by another approach. They measured the oxygen consumption of normal rat liver slices in the presence of lymph or of serum from scalded calves. The QO_2 in lymph obtained after burning was as much as 41 per cent higher than that in normal lymph. Serum had a less marked but definite augmenting tendency. These observations are not unexpected, since serum and lymph from scalded animals would undoubtedly contain increased amounts of such easily oxidizable metabolites as lactate and amino acids, or such stimulants to tissue respiration as ammonia in small amounts (as found in the blood in the present experiments).

TABLE 5

Oxygen uptake of liver slices as measured by Warburg apparatus at varying intervals after 80° 7″ scalds in rats

RAT NO.	SACRIFICED HOURS AFTER SCALD	NO. OF SAMPLES	Q_{O_2}*		
			1st hour	2nd hour	Overall
1	1	3	6.23	5.57	5.90
2	2	3	5.47	4.53	5.00
3	3	3	6.30	5.27	5.78
4	4	3	5.27	3.67	4.47
5	5	3	5.23	3.77	4.50
6	9	3	6.27	5.07	5.67
7	10	2	5.65	5.15	5.40
8	11	3	4.90	3.73	4.32
9	12	3	6.00	5.37	5.68
Normal control series.		25	5.60	4.69	5.15 ±0.10

* Cu. mm. per mgm. initial dry weight per hour. The initial wet weight of the samples was 103–106 mgm. The dry weight was 25.9–28.3 per cent of the wet weight.

It is difficult to draw conclusions from the results of the few experiments with adrenal cortical extract and normal saline (table 2). While these results are significant statistically, they do not agree too well with data from other series of rats burned at slightly different temperatures. The results are suggestive that saline therapy may be of some benefit, especially in view of the recent observations of Fox (13) and of Rosenthal (14).

Following thermal trauma there is a marked fall in adrenal total cholesterol. The fact that this fall is not observed after hypophysectomy indicates that the

trauma acts on the adrenal by way of the hypophysis. Burn shock, therefore, appears to be another of the varied types of stress which have been shown (10) to activate the hypophyseal-adrenal mechanism.

SUMMARY

Scalds were produced in white male rats of standard size by immersion in hot water at constant temperatures for definite periods of time. The following effects of the burns were recorded:

1. The plasma amino nitrogen rose in all cases, slightly after burns at 70°C., 5 seconds, markedly after burns at 80°C., 7 seconds. The increase was progressive within the time limits studied (12 hrs.).

2. The free amino nitrogen level of the livers was higher in burned than in normal rats. The ammonia level of the blood was increased in burned rats, while there was very little change in the ammonia or amide content of the livers.

3. The rate of respiration of slices from the livers of scalded rats was not significantly different from normal.

4. The adrenal total cholesterol fell to as low as one-fifth of the control value when measured as milligram per cent of adrenal weight. The decrease was definite after mild, as well as after severe, scalds. There was no change in adrenal weight. The intraperitoneal injection of adrenal cortical extract or of saline alcohol solution reduced somewhat the decrease in adrenal cholesterol.

5. Hypophysectomy done 3 days before the scalding entirely prevented the usual fall in adrenal total cholesterol following the scalds.

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THE EFFECTS OF HEPATIC ANOXIA ON THE RESPIRATION OF LIVER SLICES IN VITRO[1]

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The observation that the metabolism of liver slices from rats in hemorrhagic shock is depressed to a degree corresponding to the severity of shock (1, 2), and the suggestion that these effects on the liver are probably due to lack of oxygen, made it of interest to study the effects of anoxia uncomplicated by loss of blood on the liver *in situ*. It is possible to prepare the liver in the rat so that the sole blood supply is provided by the hepatic artery (3). In these circumstances the ability of the liver to remove amino acids from the blood is not greatly diminished. However, if anoxia is induced by clamping the hepatic artery for 60 minutes or longer, the blood amino nitrogen continues to increase for many hours after the blood supply to the organ is restored, the degree of liver failure increasing with increasing duration of anoxia. The liver appears to be able to recover completely the ability to remove amino acids from the blood if the period of anoxia is 45 minutes or less.

These observations led to a study of the effects of hepatic anoxia on the rate of respiration of rat liver tissue, *a*, after varying periods of occlusion of the hepatic artery, and *b*, after recovery from a given period of anoxia.

METHODS AND MATERIALS. Male albino rats of the Sprague-Dawley strain, weighing from 250 to 300 grams, were prepared as described previously (3). The respiration of thin slices of the liver tissue was measured in the Warburg apparatus. The general methods employed in preparing the tissue have already been described (1). In each experiment, samples of liver slices (100 mgm. wet weight) were prepared in triplicate, care being taken to make the time of preparation as uniform as possible. The tissues were incubated in phosphate-buffered physiological salt solution (pH 7.4) in an atmosphere of oxygen for one hour. The rates of oxygen uptake were calculated on the basis of the initial dry weight of liver tissue taken.

Three series of experiments were carried out. 1. In a control series of rats the liver was prepared with the hepatic artery as the sole blood supply, and the rate of respiration of samples of the liver tissues was determined at one, two, three and four hours after the operation was completed. This provided information on the effects of shifting from mainly a venous to an arterial blood supply, with its richer oxygen content. 2. In another series of preparations a portion of liver tissue was taken immediately after occlusion of the hepatic artery,

[1] The work described in this paper was done under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and Yale University.

in order to obtain an estimate of the initial rate of respiration of the tissue, and a second sample was taken at 5, 15, 30, 60 or 120 minutes after occlusion of the hepatic artery. This provided information upon the state of the tissue respiration after anoxia of increasing duration. 3. In the third series of preparations the hepatic artery was occluded for 15, 30, 60, or 120 minutes, and then released for two hours, at which time tissue samples were taken for the respiration studies, in order to see what degree of recovery was attained after each period of anoxia.

Results. The results of these experiments are summarized in figure 1. In the control series the rate of respiration of the liver slices fell slightly in the first hour (while adjustment of the circulation was doubtless taking place) and then rose steadily to a level significantly higher than the initial level at the end of three hours. This may be a consequence of the enriched oxygen supply from the purely arterial blood circulating in the organ. Its importance lies in the possibility that the estimates of recovery after anoxia may be too generous, since in the intact animal with normal circulation oxygen is provided mainly at much lower pressures via the portal vein. The rate of respiration of the liver tissue is depressed in increasing degree as the period of anoxia is increased. In 30 to 120 minutes the rate of oxygen uptake has fallen to the same level as that found in the liver tissue of rats in severe shock after hemorrhage. During the two hour period of recovery from anoxia, the liver shows considerable ability to restore its rate of respiration. In each instance the degree of recovery is related to the duration of anoxia. (The dotted lines in the figure connecting the recovery points with the corresponding anoxia points are not intended to suggest that the recovery follows a linear course, but are merely intended to make the relationship clear.)

Discussion. From figure 1 it may be seen that a large component of the liver respiration is suppressed after anoxia of short duration. The rate of oxygen uptake of the liver tissue after only 15 minutes of circulatory arrest *in vivo* is about 40 per cent of the initial rate. Whether this abrupt fall is associated with failure of one or of all of the systems that deal with the major tissue substrates remains to be ascertained. It is probably not due to damage to the cytochrome-oxidase system, since liver slices exhibiting similar low basal rates of oxygen uptake after anoxia due to hemorrhage or after anoxia *in vitro* are able to oxidize succinic acid as rapidly as normal rat liver slices (1).

In the two-hour period of recovery after release of the occluded hepatic artery an appreciable degree of repair of the liver respiration may take place. The effects of a 15 minute period of circulatory arrest are almost completely reversed in two hours after the hepatic artery is released. After longer periods of anoxia recovery is less complete. In the recovery period following 120 minutes of circulatory arrest the improvement in oxygen uptake is significant, but the level of the tissue respiration is still low—of the same order as that observed after 15 minutes of anoxia. It is possible that the functions of the systems associated with the initial large depression of liver respiration have not in this instance been restored to any appreciable extent. Perhaps it should be empha-

sized as a precaution that these experiments cannot reveal whether the principal components of the respiration after recovery are the same as those contributing to the initial rate of oxygen uptake of the tissue. The simplest hypothesis is that they are, but this assumption must be tested experimentally.

Some support for associating the improvement in liver respiration observed during the recovery period with the restoration of normal function is provided by the observations of Engel, Harrison and Long (3) on changes in blood amino

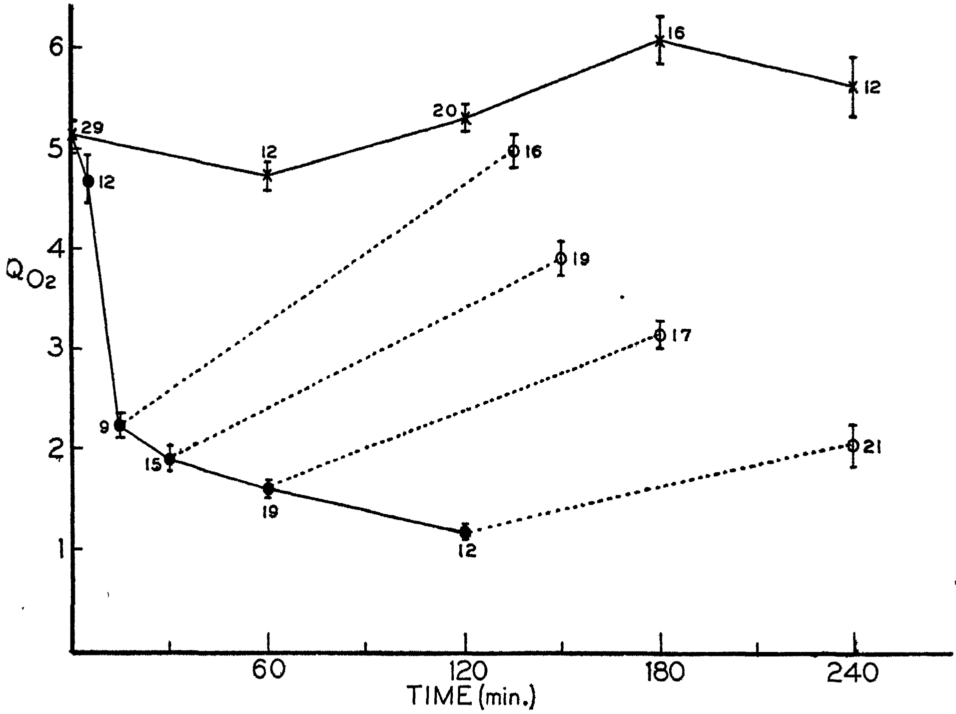

Fig. 1. Effect of hepatic anoxia *in vivo* on respiration of rat liver slices *in vitro*. *Crosses:* control observations on liver samples from operated animals. *Solid circles:* samples from livers with hepatic artery clamped for 0, 5, 15, 30, 60 and 120 minutes. *Open circles:* samples taken two hours after restoration of circulation to the liver following 15, 30, 60 or 120 minutes of hepatic anoxia. Small figures at each point indicate number of observations. Vertical bars indicate standard error of the means.

nitrogen after hepatic anoxia. After anoxia of 60 minutes' duration or less the liver removed amino acids from the blood at rates adequate to prevent any considerable increase in blood amino nitrogen above the levels attained at the time of release of the hepatic artery. There was an initial small rise during the first hour of recovery after 60 minutes of anoxia, but during the ensuing five hours the blood amino nitrogen rose only slightly more rapidly than it did during the same period in control animals in which the hepatic artery had not been occluded. After 120 minutes of anoxia the blood amino nitrogen continued to rise for several hours after the hepatic artery was released. The rate of

increase in the blood amino nitrogen in the first hour was greater than that observed in a completely eviscerated rat in the corresponding time interval, which suggests that the anoxic liver may have been adding amino acids to the blood during this time. Thereafter, the rate of increase in blood amino nitrogen diminished in each succeeding hour, until in the third and fourth hours after release of the hepatic artery the rise in blood amino nitrogen was only about one third of that observed in the completely eviscerated rat in the corresponding time intervals. The liver, initially preoccupied with substrate of hepatic origin, had clearly regained some of its ability to remove amino acids from the blood. This may be associated with the observed improvement in basal oxygen uptake of the liver tissue during the recovery period. Although the rates of respiration of the liver tissue removed at two hours after 60 or 120 minutes of circulatory arrest are low, the rates of deamination may not be depressed in the same proportion, since in liver slices from rats in severe hemorrhagic shock the rates of deamination are relatively less affected than are the rates of oxygen uptake (2).

It is probable that in addition to deamination, assimilation of amino acids by the synthesis of polypeptides and proteins also takes place in the normal liver. The failure of the liver to prevent a continuing rise in blood amino nitrogen after anoxia of more than 60 minutes' duration may in part reflect the loss, or the retarded recovery, of this anabolic function of the organ. It is worth noting that in the liver tissue of rats in severe hemorrhagic shock the rate of urea synthesis, a process requiring energy, is relatively more severely depressed than the rate of oxygen uptake (2). The oxygen consumed by the liver slices after prolonged anoxia may thus be very largely used in catabolic reactions like deamination rather than in reactions leading to the development of energy for assimilatory processes.

The experiments described in this paper comprise the first part of a study of the factors influencing the resistance of the liver to anoxia following circulatory arrest, and affecting the rate of recovery of the organ after exposure to such stress. There is now a fair amount of evidence indicating that the liver is one of the organs most severely affected in shock, in which there is an impaired blood and oxygen supply to that organ particularly. Although it cannot be said that impaired hepatic function is directly responsible for death in shock, hepatic failure may be one of the sustaining factors in shock of long duration, and the rate of recovery of liver function must certainly influence the course of recovery and of convalescence after the immediate crisis of shock has been overcome. These experiments furnish the outlines of a useful method for the study of factors influencing the recovery of the liver after periods of anoxia.

SUMMARY

1. In rats in which the sole blood supply to the liver was provided by the hepatic artery, occlusion of the artery for 5, 15, 30, 60 and 120 minutes caused a progressive fall in the rate of respiration of the liver tissue *in vitro*. After 15

minutes of anoxia the respiration rate of the slices was reduced by half, and after one hour, to about one-third the normal rate.

2. Two hours after restoration of the circulation through the liver, the respiration of the liver slices showed a degree of recovery related to the duration of anoxia. After 15 minutes of anoxia the respiration rate returned to normal in two hours, after 30 minutes of anoxia, to two-thirds the normal rate, after 60 minutes of anoxia, to about one-half the normal rate. After two hours of anoxia the amount of recovery was significant, but the rate was still very low.

3. The relationship of these findings to the ability of the liver to remove amino acids after anoxia is discussed. The experimental procedure employed is suggested as the basis for a study of the factors influencing the recovery of the liver from damage due to shock or anoxia.

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SOME ASPECTS OF THE NITROGEN METABOLISM OF LIVER TISSUE FROM RATS IN HEMORRHAGIC SHOCK[1]

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A characteristic feature of the metabolism of rats in hemorrhagic shock is a marked increase in the level of plasma amino nitrogen. This increase has been correlated with the degree of shock present as shown by the clinical symptoms, with the decrease in arterial blood pressure, with a decrease in oxygen saturation of both portal and peripheral venous blood, and with a decrease in rate of respiration of slices of liver tissue taken from animals in shock (1–3). It has also been shown that anoxia of the liver *in situ*, without loss of blood or other evidence of shock, is followed by a progressive rise in the blood amino nitrogen level, and that anoxia either *in vivo* or *in vitro* leads to a marked decrease in the rate of respiration of the liver tissue (2, 4). The increase in the level of circulating amino nitrogen in shock has been shown to be due at least in part to release of amino acids from the peripheral tissues (5). However, the association of damage to the respiratory systems of the liver with increases in blood amino nitrogen levels both in shock and after hepatic anoxia *in vivo* suggests that failure of the liver to metabolize amino acids may also be a significant feature of hemorrhagic shock.

Failure in the metabolism of amino acids by the liver might involve defects in their assimilation (protein or peptide synthesis) or in their deamination, or in urea synthesis. Some aspects of these possibilities have been examined in the following ways: 1, by determining whether there are significant changes in the concentration of ammonia in the blood and in the amounts of amino, ammonia, and amide nitrogen in the liver, in shock; and 2, by determining the rate of deamination of an amino acid, and of urea synthesis from an amino acid and from ammonium lactate by liver slices taken from animals in hemorrhagic shock.

METHODS AND MATERIALS. Fasted male albino rats of the Sprague-Dawley strain, weighing from 250 to 300 grams, were used in these experiments. In all of the experiments the animals were anesthetized with sodium pentobarbital (nembutal) in a dose of 4 mgm. per 100 grams. Shock was induced by removing from 2.8 to 3.0 per cent of the body weight of blood from the cut end of the tail in the course of an hour. The animals were sacrificed from one to two hours after the end of bleeding. No attempt was made to obtain uniformity in the

[1] The work described in this paper was done under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and Yale University.

degree of shock attained, since it was of particular interest to study the changes in nitrogen metabolism of the liver in relation to the severity of the state of shock induced. As described in an earlier paper (3), the degree of shock was judged to be mild, moderate, or severe, according to the increase in blood amino nitrogen and to rate of oxygen uptake of the liver tissue.

The general methods of preparing the liver slices for study *in vitro* have been described in another paper (3). For measurements of oxygen uptake the slices were incubated in 3 ml. of physiological salt solution containing phosphate buffer (0.017 M; pH 7.4), under an atmosphere of 100 per cent oxygen. Some observations on urea formation in this medium were also made. For most of the studies of urea synthesis and deamination the medium was buffered with bicarbonate (0.024 M, pH 7.4), and the vessels were filled with a mixture of 95 per cent oxygen and 5 per cent carbon dioxide. Each vessel contained about 100 mgm. (wet weight) of liver slices. The time of incubation was two hours. The initial dry weight of the tissues was determined on parallel samples taken at the time the slices were prepared. The data of this paper are presented in terms of the initial dry weight of the tissues taken, except where otherwise noted. In the experiments in which chemical determinations were made, initial values were obtained from samples prepared and equilibrated in the bath, along with the experimental samples, but removed and prepared for analysis at the end of the equilibration period.

Urea was determined manometrically on duplicate 1 ml. aliquots of the medium by a modification of the method of Krebs and Henseleit (6). To the fluid and slices remaining in the vessels after removal of these samples, 0.3 ml. (for phosphate-buffered media) or 0.4 ml. (for bicarbonate-buffered media) of 0.25 N sulfuric acid was added. After mixing, 0.1 ml. of 10 per cent sodium tungstate was added to precipitate the proteins, and redistilled water was added to make the final volume 2 ml. The mixed contents of the vessels were decanted and centrifuged. Determinations of total ammonia (free plus "amide" ammonia) were made on 1 ml. aliquots of the supernatant liquid as described below.

In one series of experiments on bled rats amino, ammonia and "amide" nitrogen was determined on tungstate filtrates from samples of liver tissue taken at the time of sacrifice. The samples (about 100 mgm. wet weight) were put into tared 15 ml. graduated centrifuge tubes containing 0.3 ml. of two-thirds normal sulfuric acid, weighed and pulped with a glass rod. The mixture was diluted with redistilled water, 0.3 ml. of 10 per cent sodium tungstate was added, and the volume was brought to 5 ml. with water. After mixing, the tubes were allowed to stand for about one-half hour before they were centrifuged. One-half milliliter aliquots of the supernatant fluids were used for estimations in duplicate of amino and of free ammonia nitrogen. Estimations of total easily hydrolyzable nitrogen, including both free and "amide" nitrogen, were made as described by Krebs (7). To 1 ml. aliquots of the protein-free fluid in small test tubes, 0.1 ml. of a mixture of equal parts of concentrated sulfuric acid and water was added. Hydrolysis was carried out by placing the

tubes in boiling water for 5 minutes. After cooling, the contents of each tube were neutralized with potassium hydroxide to about pH 5 and made to a volume of 2 ml. with redistilled water. Aliquots of these solutions and of blanks containing acid, alkali, and indicator were analyzed for ammonia nitrogen. The difference in ammonia nitrogen between that found directly and that found after hydrolysis was called "amide" nitrogen. It is believed to be derived mainly from glutamine, although other sources of easily hydrolyzed ammonia may contribute to it.

Ammonia was determined in all cases by the microdiffusion method of Conway (8) used in conjunction with a colorimetric estimation of ammonia based on its reaction with phenol and hypocholorite (9). In the determinations on tissue extracts, complete absorption of the ammonia was allowed to take place at room temperature. For the determination of blood ammonia the interrupted absorption technique of Conway was used. One-half milliliter samples of blood were taken from the vena cava into hypodermic syringes moistened with ammonia-free potassium oxalate solution, measured in the syringe, and delivered immediately into small Conway units containing 0.5 ml. of potassium carbonate in the outer chambers and 0.5 ml. of 0.01 N hydrochloric acid in the inner wells. At the same time similar units were prepared containing, instead of blood, either standard amounts of ammonia or redistilled water (for blanks). Absorption of ammonia was allowed to proceed at room temperature for thirty minutes under identical conditions in all units. At the end of this time the acid in each unit was rinsed into a colorimeter tube. The colors were then developed and the unknown and standard samples directly compared in the photoelectric colorimeter. Corrections were made for the amount of ammonia liberated by the alkali from the blood constituents, as described by Conway.

Blood amino nitrogen was determined on 0.2 ml. samples of whole blood taken before bleeding and at the time of sacrifice of the animal. All amino nitrogen determinations were made by the method of Frame, Russell, and Wilhelmi (10).

RESULTS. *Ammonia and amino nitrogen content of blood and liver in shock.* Observations on the level of ammonia in the blood and of ammonia, "amide," and amino nitrogen in the liver of rats in states of moderate or severe shock after hemorrhage are presented in table 1. The blood ammonia rose in all the rats in shock, the increases being generally greater in the animals showing the most distress. However, although the content of ammonia nitrogen of the blood of the animals in shock was relatively far above normal, it never rose above 1 mgm. per cent. The free ammonia content of liver is normally 2 to 4 mgm. per cent, and other tissues also contain free ammonia in concentrations equal to or greater than those found in the blood of animals in shock. It does not seem likely therefore that ammonia can have been a toxic factor in these animals unless their tissues were very much more sensitive than normal tissues to alterations in ammonia levels.

The livers of the animals in shock showed slight changes in ammonia content, the free ammonia increasing somewhat, the "amide" nitrogen falling in all but one experiment, and the total ammonia not changing significantly. These

figures suggest that if the ability of the liver to metabolize amino acids is reduced in shock, the rates of deamination and of urea formation *in vivo* must be reduced in a nearly parallel manner. The average amino nitrogen content of the livers of the rats in shock rose moderately, the concentrations being consistently higher in those animals in the more severe states of shock. Since the level of free amino nitrogen in the liver is normally about ten times that found in the plasma, and since the increases in amino nitrogen content of the livers of the animals in shock appeared in many cases to be too great to be accounted for solely by increases in the amino acid content of the circulating fluid, the extra amino nitrogen in the liver may have been in large part of hepatic origin. Precise evaluation of the increase in free amino nitrogen in the liver requires further investigation of the relationship between the levels of amino nitrogen of plasma and of the liver in normal animals as well as in animals in shock.

TABLE 1

Ammonia and amino nitrogen of blood and liver of rats in shock

	NO. OF OBS.	BLOOD AMMONIA NITROGEN	BLOOD AMINO NITROGEN	LIVER AMMONIA NITROGEN			LIVER AMINO NITROGEN
		mgm. per cent	*mgm. per cent increase*	*mgm. per cent** Free	*mgm. per cent** Total	*mgm. per cent** "Amide"	*mgm. per cent**
Normal rats	7	0.05	0	2.5 (1.8–4.0)	9.8 (7–16)	7.3 (4.6–12.1)	55 (47–62)
Rats in hemorrhagic shock	9	0.56 (0.30–0.81)	+7.9 (+3.0–+12.1)	4.0 (2.8–5.3)	8.1† (5–16)	4.1† (1.5–13.2)	66 (57–74)

* Based on wet weight.

† These averages include one very high value.

Deamination and urea synthesis. The effects of hemorrhage on the ability of the liver to deaminate amino acids and to synthesize urea, *a*, in the presence of an amino acid; *b*, with ammonia and lactate as substrates, and *c*, in the presence of ammonia, lactate and ornithine, were studied in two series of experiments.

In the first series, liver slices were incubated with 0.04 M dl-alanine for two hours. Urea and total ammonia nitrogen were then determined on aliquots of the medium. Initial values were determined from parallel samples taken at the beginning of the incubation period. The sum of the increases in these quantities provides an estimate of the amount of deamination that has taken place during the experiment. Some of the experiments on liver slices from normal rats were carried out in phosphate rather than in bicarbonate buffer in order to determine the effect of the medium on the ability of the tissue to deaminate the amino acid. All of the observations are summarized in table 2. Both urea synthesis and deamination are smaller in phosphate buffer than in bicarbonate buffer. All of the experiments on liver tissue from bled rats were

therefore carried out in bicarbonate buffer. The initial amounts of urea and ammonia nitrogen present in slices from normal and from bled rats are similar, and there are also no differences between the samples incubated without substrate. In the presence of dl-alanine, the amount of urea formed decreases with increasing severity of shock. The amount of deamination decreases also, but to a smaller extent; there is therefore an accumulation of ammonia in the system during incubation.

TABLE 2

Deamination and urea synthesis from dl-alanine (0.04 M) by liver slices from normal rats and from rats in hemorrhagic shock

(All figures are expressed in terms of mgm. N per gram initial dry weight)

DESCRIPTION	NUMBER OF OBSERVATIONS	UREA N	AMMONIA N	TOTAL N (UREA + AMMONIA)
Initial levels:				
Normal rats:				
Phosphate medium	2	0.70	1.05	1.75
Bicarbonate medium	5	0.25	0.39	0.59
Bled rats:				
Bicarbonate	9*	0.46	0.44	0.90
No substrate: increase over initial levels:				
Normal rats:				
Phosphate	1	0.23	0.01	0.22
Bicarbonate	5	0.52	0.04	0.56
Bled rats:				
Bicarbonate	9*	0.56	0.05	0.61
dl-alanine (0.04 M): increase over initial levels:				
Normal rats:				
Phosphate	8	1.16	1.72	2.88
Bicarbonate	16	3.11	0.45	3.56
Bled rats: bicarbonate:				
I. Mild shock	3	1.84	0.33	2.27
II. Moderate shock	9	1.28	0.68	1.96
III. Severe shock	12	0.42	1.26	1.68

* Samples from all classes of bled rats combined, since there were no significant differences between them.

The relation of these changes to each other and to the rate of oxygen uptake of the liver tissue (in phosphate buffer in the presence of glucose) and the increase in blood amino nitrogen, with increasing severity of shock, is illustrated in figure 1. At first, the decline in deamination and in urea synthesis is similar, and is proportionately greater than the decrease in oxygen uptake. In moderate and severe shock, however, deamination is not much further diminished, whereas urea synthesis continues to fall sharply, to a greater degree than the oxygen

consumption. This comparison between the rates of respiration and of urea synthesis may be too conservative, since the rates of oxygen consumption observed were basal rates with glucose as substrate. The rate of oxygen uptake of liver tissue is increased in the presence of dl-alanine, and although the oxygen uptakes could not be measured in the bicarbonate medium, it is fairly certain, in view of the observed deamination rates, that they were increased over the basal rates, even in samples from animals in severe shock. The observations tend to suggest that a process requiring energy might be more seriously affected in a tissue damaged by stress than the degree of failure in the oxygen consumption of the tissue would indicate.

In the second series of experiments urea synthesis from ammonia (0.01 M) and lactate (0.02 M dl), with and without catalysis by ornithine, was studied

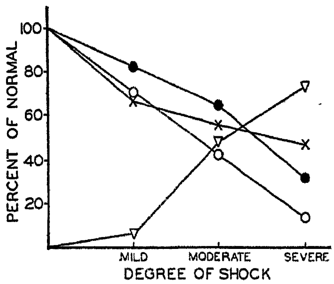

Fig. 1

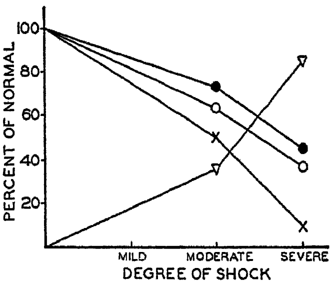

Fig. 2

Fig. 1. Percentage changes with increasing severity of shock (normal = 100 per cent) in blood amino nitrogen (▽), rate of oxygen uptake of liver slices in phosphate buffer with 0.2 per cent glucose (●), urea nitrogen synthesized (○) and total nitrogen arising from deamination (×) by liver slices in bicarbonate buffer.

Fig. 2. Percentage changes with increasing severity of shock (normal = 100 per cent) in blood amino nitrogen (▽), rate of oxygen uptake of liver slices in phosphate buffer with 0.2 per cent glucose (●), and urea nitrogen synthesized from ammonia and lactate (×) and ammonia, lactate, and ornithine (○) by liver slices in bicarbonate buffer.

in order to determine the maximum capacity of liver slices from normal and from bled rats to form urea from ammonia. These observations are summarized in table 3. The initial values for urea nitrogen in samples from normal and from bled rats are variable and not significantly different from one another. With increasing severity of shock smaller amounts of urea are synthesized. The catalytic effect of ornithine on the synthesis is evident in slices from rats in severe shock. The increases due to ornithine are rather smaller with increasing severity of shock, and marked differences in rate of synthesis persist despite the presence of the catalyst.

These observations are compared in figure 2 with the changes in rate of oxygen uptake (in phosphate buffer, with 0.2 per cent glucose) and with the increases in blood amino nitrogen that take place with increasing severity of shock. (Although no animals in mild shock were observed in this series, the scale of fig. 2 is, to facilitate comparison, the same as that of fig. 1.) The pattern of the

two figures is in most respects the same. With increased degree of shock, the failure in urea synthesis, in both the catalyzed and the uncatalyzed reaction, is proportionately greater than the fall in the basal rate of oxygen uptake of the liver tissues. The observations in both series indicate that the ability of liver slices from rats in hemorrhagic shock to synthesize urea is not limited by the rate of deamination in such tissues, or by loss of their response to the catalytic effect of ornithine, or by their rates of oxygen uptake. The diminished respiration, however, appears to be accompanied by a somewhat greater proportionate fall in the intensity of processes supplying energy for urea synthesis.

Discussion. These observations provide additional evidence of impaired liver function in the rat in hemorrhagic shock. They are consistent with the view that the rise in blood amino nitrogen observed in shock is due in part to a failure of the liver to remove amino acids, either by assimilation or by deamination and urea synthesis. They also provide indirect support for the view that the increase in circulating amino nitrogen in shock is due in part to release of

TABLE 3

Urea synthesis in liver slices from normal rats and rats in hemorrhagic shock, in the presence of sodium lactate (0.02 M), and ammonium chloride (0.01 M), with and without ornithine (0.002 M)

Urea nitrogen (mgm. per gram initial dry weight)

DESCRIPTION	INITIAL	INCREASE OVER INITIAL	
		Ammonia, lactate	Ammonia, lactate, ornithine
Normal (8)	0.66	2.74	6.29
Bled: moderate shock (10)	1.45	1.43	3.97
Bled: severe shock (4)	1.00	0.30	2.30

amino nitrogen from the peripheral tissues, since the blood amino nitrogen increases rapidly at a time when deamination and urea synthesis are still taking place at moderate rates in the liver. The capacity of the liver for deamination and urea synthesis is large, and it may be expected that in the intact animal these processes will continue at appreciable rates even in relatively advanced stages of shock. The assimilation of amino acids by the liver may, however, be more seriously impaired, as suggested by the observation that the increases in liver amino nitrogen may be partly of hepatic origin.

The question may be raised whether, with respect to deamination, these data indicate the real degree of failure of the liver in shock. The substrate employed comprised both optical isomers of alanine, and although these two forms are both deaminated by rat liver slices, according to Krebs (11) the unnatural isomer is deaminated at a rate several times greater than the natural isomer. If the fall in rate of deamination in shock were due to a selective failure of the system deaminating the natural isomer, while the other system remained relatively unaffected, then the ability of the liver to remove circulating amino acids would be more depressed than the data reveal. The observation that ammonia does

not accumulate in the liver *in vivo* with increasing severity of shock, when the capacity for urea synthesis (as judged from the *in vitro* experiments) is progressively diminished, suggests that the ability of the liver to deaminate amino acids of the natural series is in fact more seriously affected than the data on deamination *in vitro* indicate. These experiments might profitably be repeated with one or more amino acids of the natural series.

The failure of urea synthesis with increasing severity of shock is interesting because this is one of the characteristic energy-using reactions of liver tissue, and it therefore provides an opportunity of correlating the utilization of oxidative energy with the oxygen consumption of the tissue. Figures 1 and 2 indicate that there is a rough correlation between the basal rates of oxygen uptake and the rates of urea synthesis by the liver slices. They also show that urea synthesis is, at each stage, relatively more depressed than the rate of respiration, so that a fall in oxygen uptake may indicate a decrease in a reaction using energy, but it is not necessarily indicative of the degree to which that process may be impaired. If, as seems probable, this relationship applies to other energy-using reactions than urea synthesis (for instance, the assimilation of amino acids), it may mean that the integrity of a tissue can be in considerable jeopardy without there necessarily being any clear reflection of this critical situation in a corresponding failure in rate of oxygen consumption.

SUMMARY

1. In rats in shock after hemorrhage the ammonia content of venous blood was increased. The levels attained, although far above normal, did not indicate that ammonia was a toxic factor in these conditions. The livers of these animals showed slight changes in free, "amide," or total easily hydrolyzable ammonia, as determined in tungstic acid filtrates. The free amino nitrogen content of the liver tissue was high in rats in shock, the increase appearing to be in part of hepatic origin.

2. The rate of deamination of dl-alanine by liver slices taken from rats in shock after hemorrhage was decreased, but not to as great an extent as was the basal oxygen uptake of the slices.

3. The rate of synthesis of urea by the liver slices from dl-alanine or from ammonium lactate was progressively diminished with increasing severity of shock. The rate was affected more sharply than was the basal respiration rate, synthesis being nearly abolished in the slices from rats in severe shock.

4. Urea synthesis from ammonium lactate was increased in rate by catalytic concentrations of ornithine in liver slices taken both from normal and from bled rats. However, the increases in rate due to ornithine were smaller in the tissues from rats in shock, and marked differences in rates still persisted in the presence of ornithine.

5. The evidence supports the conclusion that in shock after hemorrhage in the rat, deamination and urea synthesis in the liver are progressively depressed. The rise in blood amino nitrogen in shock is probably not due alone to the failure of the liver to catabolize amino acids, but is also due to failure of the liver

to assimilate amino acids and to an increased contribution of amino nitrogen from the peripheral tissues.

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THE EFFECTS OF ANOXIA AND OF HEMORRHAGE UPON THE METABOLISM OF THE CEREBRAL CORTEX[1] OF THE RAT

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Elsewhere some of the effects of anoxia and of hemorrhage on the metabolism of slices of rat liver and kidney tissue have been described (1–3). In this paper a report is made of similar experiments on slices of rat cerebral cortex. The work done falls into four sections: 1. A determination of the conditions for obtaining uniform, steady, and reproducible rates of oxygen uptake in samples of tissue from the same rat brain and from different brains. 2. A study of the effects of increasing periods of anoxia *in vitro* on the rates of oxygen uptake of brain slices in the presence and in the absence of glucose. 3. A series of observations on the oxygen uptake of brain slices from rats exhibiting the first acute symptoms of hemorrhagic shock. 4. A study of the metabolism of brain tissue from rats in the terminal stages of shock induced by the standard procedure to be described by Sayers, Sayers, and Long (4). In the latter experiments, determinations of changes in lactate, and in amino, ammonia and "amide" nitrogen were made and compared with like observations upon a comparable series of normal rat brains. In some of these experiments the rates of respiration of slices of the liver tissue were determined and compared with those obtained from an earlier series of rats in hemorrhagic shock.

Methods and materials. The general methods used in the experiments were as follows. The medium in which the brain slices were incubated was Krebs' (5) physiological saline, phosphate buffered, pH 7.4, modified to contain 5 mgm. per cent of calcium, rather than the 10 mgm. per cent originally proposed. No precipitate of calcium phosphate is formed in this mixture. The volume of medium used was in every instance 2 ml., the atmosphere was, except where otherwise stated, 100 per cent oxygen, the temperature 37.5°, and the rate of shaking of the vessels was never less than 125 double excursions (6 cm.) per minute. Fasted male albino rats of the Sprague-Dawley strain were used throughout these experiments. The animals, unanesthetised, were decapitated by a single sharp stroke of heavy autopsy scissors. The brain was quickly removed and kept in a cold chamber during the preparation of slices. The whole cerebral cortex was sliced, the slices being handled with glass implements and stored in a cold moist chamber until they were distributed to the vessels. Samples of from 70 to 80 mgm. were weighed on a glass hook on the

[1] The work described in this paper was done under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and Yale University.

torsion balance and placed in the vessels. Parallel samples were weighed and taken for the estimation of dry weight. After distribution of the tissue, the vessels were mounted, gassed, and placed in the water bath for a ten-minute equilibration period before the addition of substrate was made and the measurements of oxygen uptake were begun. The time of preparation of the brain tissues was standardized as far as possible. As a rule, the first measurements of respiration were begun about 40 minutes after the death of the animal. Values of the rate of oxygen uptake are presented in terms of cubic millimeters per milligram of initial dry weight of tissue per hour.

In the experiments in which chemical determinations were made, the vessels were removed from the bath, wiped free of external moisture, and detached from the manometers. Four-tenths milliliter of 0.25 N sulfuric acid was run into each vessel, and the mixed contents were decanted into a 15 ml. graduated centrifuged tube. Each vessel was washed three times with 1 ml. of re-distilled water, the washings being added to the centrifuge tube. One-tenth milliliter of 10 per cent sodium tungstate was then added to the mixture in the centrifuge tube, the volume was made up to 6 ml. with redistilled water, and the contents of the tube were vigorously mixed with a glass rod and allowed to stand until the precipitate had settled. The samples were centrifuged, and the clear supernatant liquids were decanted into test tubes and stored in the refrigerator until the time of analysis.

Lactate was determined by the method of Barker and Summersen (6) and amino nitrogen by the method of Frame, Russell, and Wilhelmi (7). Ammonia nitrogen (free and "amide") was determined as described in another paper of this series (2). The "amide" or easily hydrolyzable ammonia is believed to be derived mainly from glutanine.

RESULTS. 1. *The respiration of normal rat cerebral cortex.* Four factors are important in obtaining reproducible rates of respiration in samples of brain slices from the same rat and from a series of different rats: *a*, uniformity in the time of preparation of the tissues; *b*, the presence of calcium in the medium; *c*, the presence of glucose as substrate; *d*, the selection of initial dry weight as the basis for calculating the rates of respiration. The metabolic activity of the brain tissue declines continuously from the time of its removal from the animal. It may be stabilized at any point by the addition of glucose. Hence a uniform time of preparation is necessary if the measured initial rates of oxygen uptake in samples from different brains are to be comparable. In the absence of calcium, the initial rate of respiration with glucose is about 30 per cent higher than in the presence of calcium, but the rate declines continuously until, in 3 hours, it is at the level of the initial steady rate exhibited by slices incubated with both calcium and glucose. An unrestrained, declining respiration of damaged cells appears to be superimposed upon the steady respiration of the intact cells of the slices. In the absence of glucose, the course of the respiration is the same with and without calcium in the medium; it falls in a linear fashion for 2 hours, and thereafter more slowly. The addition of glucose does not bring about recovery to the initial rate but only stabilizes the rate at the level reached at the time of the addition.

Brain slices fragment easily. After 2 or 3 hours of incubation there are many small particles in the medium, and there is a considerable amount of protein in solution. The recovery of all the tissue, or even of a uniform proportion of it, for determinations of dry weight at the end of the experiment is difficult. This difficulty may be avoided by basing the measurements of oxygen uptake upon the dry weight of tissue put into the vessels at the beginning, calculated from the wet weights of the tissues and from samples taken at that time for dry weight determination. In this series of experiments the per cent of dry weight of the brain slices made in this way was quite uniform. In a series of 52 brains, the mean dry weight was 20.73 ± 0.12 per cent. No sig-

TABLE 1

The course of the respiration of normal rat brain slices in the presence and absence of calcium and of glucose

EXPERIMENTAL CONDITIONS	Q_{O_2} (CU. MM. PER MGM. OF INITIAL DRY WEIGHT PER HOUR)			
	½ hr.	1 hr.	2 hr.	3 hr.
No substrate, calcium	(32)* 6.97 ±0.15†	(30) 5.72 ±0.12	(28) 2.41 ±0.07	(10) 1.36 ±0.09
Glucose (0.2%) calcium	(32) 9.05 ±0.15	(32) 9.23 ±0.14	(32) 9.32 ±0.11	(16) 9.04 ±0.17
No substrate, no calcium	(2) 7.55	(2) 6.20	(2) 2.30	(2) 1.40
Glucose (0.2%) no calcium	(10) 12.12 ±0.04	(14) 11.86 ±0.29	(14) 10.41 ±0.21	(14) 9.09 ±0.17
In air: no substrate, calcium	(2) 7.25	(2) 6.15	(2) 2.95	(2) 1.70
In air: glucose (0.2%) calcium	(10) 8.13 ±0.19	(10) 8.35 ±0.11	(10) 8.37 ±0.10	(10) 8.55 ±0.13

* Numbers in parentheses indicate the number of inidividual samples.
† Standard error of the mean.

nificant differences were observed between brains from normal rats and those from rats in shock. Gross irregularities in rates of oxygen uptake, which have been one of the chief complaints against reported experiments with brain slices in the past, were not encountered in these experiments. It was therefore possible to anticipate that any differences in the rates of respiration of brain slices from normal rats and from rats in hemorrhagic shock could be treated with confidence as being real. Data illustrating the main points of this section are summarized in table 1 and figure 1.

2. *The effects of anoxia upon the respiration of normal rat cerebral cortex.* Since one of the consequences of severe hemorrhage might be to deprive the brain of an adequate oxygen supply, it was thought of interest to determine the effects

of lack of oxygen for varying periods upon the rate of respiration of brain slices *in vitro*. In these circumstances it is possible to determine, without other complications, the resistance of the tissue to lack of oxygen, the effects of addition of substrate upon the recovery of the tissue after exposure, and the effects of substrate upon resistance to exposure to anoxia. The arrangements for these experiments were as follows. Brain slices from normal rats were prepared and distributed in the vessels in the usual manner. All of the vessels were then filled with nitrogen and mounted in the bath. At 10, 15, 20 or 30 minutes from this time, four vessels were removed, filled with oxygen, and returned to the bath. After 10 minutes for temperature equilibration, the manometers were closed, substrate was tipped in, and the measurement of oxygen uptake was started. Of the four vessels in each set, one pair contained no substrate and the other contained glucose in a final concentration of 0.2 per cent. In another series, the same procedure was followed, except that the addition of substrate was

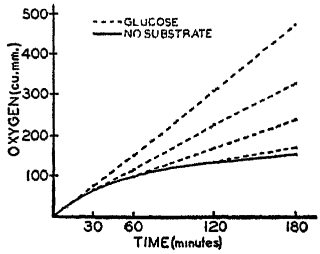

Fig. 1

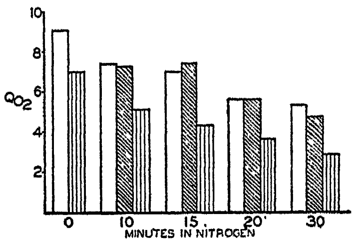

Fig. 2

Fig. 1. Effects of delayed addition of substrate to slices of rat cerebral cortex. Glucose (0.2 per cent) was added at 0, 30, 60 and 120 minutes.

Fig. 2. Effects of anoxia on the initial rate of respiration of rat brain slices. White columns: glucose (0.2 per cent) added at beginning of incubation; diagonal hatching: glucose added at end of period in nitrogen; vertical hatching: no substrate.

made at the beginning of the period of incubation in nitrogen. The course of the respiration was followed in each case for 3 hours from the beginning of the experiment. There were no notable differences between normal and anoxic tissue in the shapes of the curves obtained, so that the initial respiration rates are fairly representative of the differences in rates maintained throughout the experimental period. The rates of respiration for the first half-hour are presented in figure 2.

With increasing time of exposure to nitrogen the subsequent respiration was increasingly depressed. In the absence of substrate the rate after 30 minutes' exposure to nitrogen was equivalent to the rate after 2 hours in oxygen. In each instance the rate of respiration was increased, although not restored to normal, by the addition of glucose at the end of the period in nitrogen. In this respect, the effect of anoxia is different from that of deprivation of substrate. The presence of glucose during the period of exposure to nitrogen did not appear to increase the resistance of the tissue to anoxia. These effects of exposure to nitrogen may be compared with the effects of incubating brain slices

in air (table 1). The rates of respiration were only slightly less in air than they were in 100 per cent oxygen. Normal brain tissue appears to be rather resistant to partial lack of oxygen, but evidently it is permanently damaged by more profound anoxia over any period of time.

3. *The effects of hemorrhage (without re-transfusion) upon the oxygen uptake of rat cerebral cortex in vitro.* A group of four rats was bled according to the initial stage of the standard procedure outlined by Sayers, Sayers and Long (4). The animals were suspended in harness, and 2.6 per cent of the body weight of blood was removed from the cut end of the tail in the course of an hour. The animals were left in harness until clear indications of distress were exhibited. They were then decapitated, the brain was quickly removed, and slices were prepared for measurement of the oxygen uptake as outlined above. Of the four animals two were moribund at the time of sacrifice; the remaining pair were not in an acutely depressed state. There was very little bleeding on decapitation, and the brain itself was almost bloodless, so that the major divisions of the cerebrum which are usually clearly outlined by blood vessels in the surrounding membranes, were difficult to see. The results of the respiration experiments with this group are presented in table 2. Only the two rats exhibiting severe symptoms (nos. 1 and 2) showed a marked increase in plasma amino nitrogen and some, but not marked, failure in rate of respiration, both in the absence of substrate and with glucose. The fall in rate of oxygen uptake of the brain slices in these animals is proportionately very much less than the degree of failure in rate of oxygen uptake of liver slices taken from animals exhibiting comparable increases in plasma amino nitrogen after severe hemorrhage (1).

4. *The metabolism of rat cerebral cortex from animals after hemorrhage with repeated re-transfusion insufficient to permit recovery ("terminal shock").* It was thought that the changes exhibited by the pair of rats in the acute initial stages after hemorrhage might be accentuated in rats that had been subjected to the standard procedure of bleeding and careful re-transfusion described by Sayers, Sayers and Long (4). In the course of their experiments a number of brains from such animals in the terminal stage were made available for study. The first brain studied did have a low rate of respiration. However, in this experiment the rat had been anesthetised with nembutal in order to facilitate the removal of blood for a number of analyses. The effects of nembutal (4 mgm. per 100 gm. body weight) were therefore determined on a pair of normal rat brains for comparison (table 3). Nembutal did not appear to have significant effects on the respiration of brain slices from normal rats. However, it could have depressed the metabolism of brain tissue from rats in the terminal stages of the standard bleeding procedure; and this possibility seems to be borne out by the series of observations next recorded.

A series of eight rats in "terminal shock," from which the brains were removed after decapitation without anesthesia, was next studied. In these experiments observations were also made upon the changes during incubation in lactic acid and amino, ammonia, and "amide" nitrogen, and these were compared with

similar observations made upon brain slices from a series of 6 normal rats. The data are summarized in tables 4 and 5. Only 2 out of 8 rats in "terminal shock" exhibited a lowered rate of respiration of the brain tissue; in these, the initial

TABLE 2

Respiration of brain slices from bled rats

DESCRIPTION	Q_{O_2} (CU. MM. PER MGM. DRY WEIGHT PER HOUR)			
	½ hr.	1 hr.	2 hr.	3 hr.
No substrate:				
Normal (from table 1)	6.97	5.72	2.41	1.36
Bled: Rat 1	5.90	4.80	2.15	1.10
2	6.05	5.00	2.50	1.45
3	6.90	5.90	2.55	
4	7.20	6.05	2.95	
Glucose:				
Normal (from table 1)	9.05	9.23	9.32	9.04
Bled: Rat 1	8.70	8.80	8.45	7.60
2	8.10	8.35	8.40	8.35
3	8.50	8.75	9.00	
4	9.75	9.80	9.75	

Changes in plasma amino nitrogen: Rat No. 1: + 8.3 mgm. per cent.
2: + 8.4 mgm. per cent.
3: − 1.9 mgm. per cent.
4: + 1.9 mgm. per cent.

TABLE 3

Respiration of brain slices from anesthetized rats

DESCRIPTION	Q_{O_2}		
	½ hr.	1 hr.	2 hr.
No substrate:			
Normal (unanesthetized)	6.97	5.72	2.41
Normal (anesthetized)	7.95	6.55	2.90
Bled (anesthetized)	3.35	3.00	1.75
Glucose:			
Normal (unanesthetized)	9.05	9.23	9.32
Normal (anesthetized)	9.82	9.70	9.45
Bled (anesthetized)	7.60	7.25	6.85

brain lactate concentration was very low, but the aerobic glycolysis rates were normal. Since all the other measurements were quite uniform for both series of rats, only the mean values for the observations of changes in brain lactate and amino, ammonia, and amide nitrogen are recorded in table 5.

In 5 rats of this series duplicate samples of liver tissue were also taken for the determination of rates of oxygen uptake. They uniformly showed depressed rates of respiration similar to those already observed (1) in liver tissue from rats in moderate to severe states of shock (table 6).

DISCUSSION. Under the conditions of these experiments no consistent general effects of hemorrhage on the metabolism of rat cerebral cortex *in vitro* have been observed. Although the train of symptoms in the terminal stages of the standard procedure suggests a marked disturbance of the central nervous system, the behavior of the brain *in vitro* does not offer any clues to the processes which are failing. There is obviously no accumulation of lactic acid or ammonia

TABLE 4

Respiration of brain slices from rats in "terminal shock"

DESCRIPTION	Q_{O_2}		
	½ hr.	1 hr.	2 hr.
No substrate:			
Normal (12 obs.):	6.28 ±0.10*	5.19 ±0.12*	2.19 ±0.05*
Terminal shock: Rat 5	7.80	6.65	2.85
6	7.40	6.40	3.05
7	6.15	5.10	2.20
9	6.4	5.1	2.2
10	5.4	4.7	2.5
11	3.4	3.1	1.8
12	2.7	2.4	1.3
Glucose:			
Normal (12 obs.):	8.67 ±0.29*	9.00 ±0.28*	9.30 ±0.24*
Terminal shock: Rat 5	9.80	9.95	9.55
6	9.35	9.55	9.10
7	9.00	9.30	8.80
8	8.40	8.35	7.65
9	9.5	9.7	9.5
10	8.7	8.6	8.3
11	6.7	7.1	6.8
12	6.2	6.5	5.8

* Standard error of the mean.

in toxic concentrations, nor is there any noticeable disturbance in glycolysis or in nitrogen metabolism of brain slices from rats in severe shock. It is possible that in "terminal" hemorrhagic shock the brain is protected from anoxia until almost the moment of death. In the animals prepared by the standard procedure of bleeding and retransfusion it was noticed upon opening the skulls that the brains and surrounding membranes were richly engorged, in marked contrast to the bloodless pallor of the brains in the group of rats that were bled without retransfusion. This seems to indicate that in the procedure for producing "terminal shock" there is no very long period of oxygen lack in the brain. That anoxia of other organs is produced is shown by the characteristic low rate

TABLE 5

*Lactate and amino, ammonia and "amide" nitrogen of slices of rat brain before and after incubation**

DESCRIPTION OF RATS	NORMAL				"TERMINAL SHOCK"			
	No. of observ.	Initial†	Final	Change	No. of observ.	Initial†	Final	Change
Lactate—mgm. per gram								
		mgm. per gm.	*mgm. per gm.*	*mgm. per gm. per hr.*		*mgm. per gm.*	*mgm. per gm.*	*mgm. per gm. per hr.*
No substrate	6	4.1			7	3.0		
	12		0.95	−1.6	10		0.72	−1.0
Glucose	12		38.5	+15.6	10		43.1	+15.7
Amino nitrogen—mgm. per gram								
No substrate	6	2.6			7	2.8		
	12		2.9	+0.35	10		3.2	+0.35
Glucose	12		3.6	+1.07	10		4.0	+1.24
Ammonia nitrogen—mgm. per gram								
Total ammonia	4	0.77			7	0.88		
No substrate	8		1.58	+0.81	10		1.89	+1.01
Glucose	8		1.12	+0.35	10		1.51	+0.63
Free ammonia	3	0.41			7	0.48		
No substrate	6		1.50	+1.09	10		1.67	+1.19
Glucose	12		1.02	+0.61	10		1.22	+0.74
"Amide" nitrogen	3	0.36			7	0.40		
No substrate	6		0.08	−0.28	10		0.22	−0.18
Glucose	8		0.10	−0.26	10		0.29	−0.11

* All values are referred to the initial dry weights of the samples.

† Samples taken after equilibration, immediately before beginning of measurement of respiration.

TABLE 6

Respiration of liver tissue from rats in "terminal shock"

(Glucose 0.1–0.2 per cent present)

DESCRIPTION	Q_{O_2}		
	½ hr.	1 hr.	2 hr.
Normal (28 observ.)		5.40	4.51
Terminal shock: Rat 8	3.10	3.20	2.75
9	3.15	3.25	2.65
10	2.40	2.50	2.15
11	4.25	4.40	4.00
12	2.15	2.10	1.70

of respiration of liver slices obtained from these same animals. If, however, the circulation to the brain were even moderately well preserved, marked effects on the subsequent respiration of the tissue would not be expected, in view of the

relatively small effects of partial anoxia (respiration in air) and of complete anoxia for very brief periods which were observed here in the *in vitro* experiments. The data of Craig and Beecher (8), who made observations on respiration and glycolysis in slices of cat brain at varying oxygen tensions, are also consistent with this view, since only moderate differences in oxygen uptake and lactic acid production were encountered in changing from 100 per cent oxygen to air.

On the other hand, it is possible that alterations did take place in the metabolism of the brain which were not reflected in the behavior of the surviving slices. The metabolism of only a relatively small part of the brain rather than of the whole brain may have been affected. The metabolism of brain slices in phosphate buffered media may possibly be altered in such a way as to obscure differences in the metabolism of tissues from normal rats and from rats in hemorrhagic shock; but differences due to anoxia *in vitro* were clearly shown in these circumstances. The most likely possibility would be that, while alterations in the rates of respiration and of glycolysis may not be seen in tissue from animals in shock, there may be a disruption in the effective supply of energy from these processes or in the energy-using systems in these tissues.

SUMMARY

1. Reproducible initial rates of respiration in the absence of substrate and steady rates of respiration for at least three hours in the presence of glucose may be obtained in slices of rat cerebral cortex, provided *a*, that the medium contains calcium; *b*, that the initial dry weight of the tissue is chosen as the basis for calculating the rates of oxygen uptake, and *c*, that the time of preparation of the tissue is carefully standardized.

2. The rate of respiration of brain slices from normal rats in the absence of glucose was depressed to an increasing degree with increasing duration of anoxia after incubation in nitrogen for 10, 15, 20 and 30 minutes. The rate of respiration was increased, but not to normal, if glucose was added either just before or at the end of the period of anaerobiosis. The respiration of brain slices in air was very nearly the same as that in oxygen.

3. In two of a group of four rats which had been bled 2.6 per cent of their body weight in an hour, in which there occurred an increase in plasma amino nitrogen of about 8 mgm. per cent, the respiration of cerebral cortex slices was somewhat low. The brain tissue of the other two rats, in which no change in plasma amino nitrogen occurred, respired at normal rates.

4. A series of nine rats in the terminal stages of a standard procedure for producing "irreversible shock" was examined. In one, which had been anesthetized with nembutal, the oxygen uptake was severely depressed, although the same dose of nembutal had no effect on the respiration of brain slices from normal rats. In the remaining eight rats of the series, which were killed by decapitation without anesthetic, the rates of respiration of the brain slices were normal in every instance but two, and in these two, the rates were only slightly depressed. In this series observations were made of the lactic acid and of amino, ammonia and "amide" nitrogen of brain slices before and after the period of respiration. No significant differences were found between these observations

and those made on brain slices from a comparable group of normal rats. The rate of respiration of slices of liver tissue from animals in terminal shock was characteristically depressed.

No consistent effects of shock after hemorrhage on the metabolism of brain were observed in this series of experiments. It may be that the brain is protected from anoxia better than other tissues during hemorrhagic shock, so that marked effects are not to be expected; or there may be metabolic processes affected which are not measured in these circumstances.

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THE ACTION POTENTIALS OF THE STOMACH

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The potential changes associated with the conduction of impulses in muscle and nerve can be interpreted only if they are led off from small regions of the tissue. This is accomplished most satisfactorily by recording monophasic potentials or by recording the potentials in the intact tissue with differential electrodes. In the study of the stomach only the second of these methods is practical. However, as shown by Cole and Curtis (7), the differential potential is the first derivative of the monophasic potential provided the distance between the leads is appreciably less than the length of the active region. It is possible, therefore, to derive the monophasic potential mathematically.

It is important to realize that the maximal distance between the leads permissible for recording differential potentials is not the same for different tissues and that it must be the smaller, the shorter each wave of excitation. In smooth muscle the waves are shorter than in any other tissue studied. Therefore, the leads should be very small and their distance should be preferably less than one millimeter. Some events of the action potential of smooth muscle, like the rising phase or spike potentials, take up a width of less than one millimeter (5) and such events cannot be resolved accurately with the methods available at the present time. For illustration, the potentials of the stomach of the guinea pig may be mentioned (p. 697). During each peristaltic wave, as many as twenty spikes can be observed. Since the active region, as seen by direct observation, is less than one centimeter wide, the spikes are spaced on the muscle at a distance of less than half a millimeter. The failure to recognize this point, the use of large electrodes separated one centimeter or more, largely explains why previous investigators obtained widely disagreeing results and why their records show such marked variability.

Records obtained by Alvarez and Mahoney (2) with a slow galvanometer show no resemblance to those which will be described below. Richter (9) using a string galvanometer and monopolar (not monophasic) leads recorded during each contraction several slow waves, beginning with a positive deflection. Two of these waves undoubtedly are identical with the R and T wave described below.

METHOD. Dogs (5–7 kgm.), cats and guinea pigs anesthetized with nembutal were used. The abdomen of the animal was inside a heated and humidified chamber. The stomach was exposed by a longitudinal incision. In cats and dogs, usually a rubber balloon filled with water was introduced into the stomach. In early experiments, thin cotton wicks soaked in Ringer's solution were used as leads. The differential electrode illustrated in figure 1 was found much more satisfactory because the size and distance of the leads could be reduced to a

minimum and because movement artifacts were avoided more readily. The electrode consists of two capillary calomel electrodes of about 0.3 mm. inside diameter arranged so that the distance of the center of the two electrodes is about 0.6 mm. The potential difference between them is very small and constant. The electrode is permanent, provided that drying is prevented.

It was assumed that movement artifacts are insignificant if potential fluctuations synchronous with respiration are absent, because passive movements due to respiration are usually much larger than those caused by the active movements of the stomach. In all graphs, upward movement indicates negativity orad. An oscillograph or a mechanical recorder, both driven by a direct coupled amplifier, were used.

RESULTS. In dogs, differential records of a peristaltic wave invariably show three waves which can be designated as R, S and T waves (fig. 2). They are

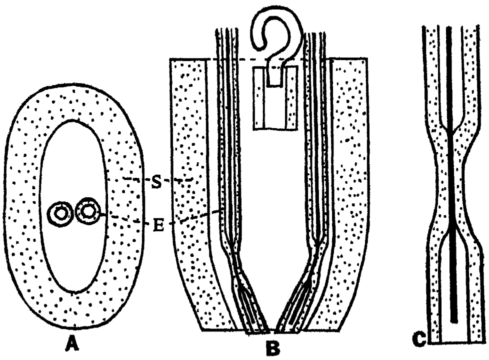

Fig. 1. Diagram of differential electrode. A: view from below showing surface in contact with muscle; B: side view; C: capillary calomel electrode; A and B enlarged 5 times, C 9 times. *S*, shell made of glass or enameled brass. *E*, capillary electrode. Each capillary electrode contains a Pt-wire gauge 31 which is fused into the glass capillary. The part of the wire in the lower chamber, making contact with the muscle, is mercury-plated and filled with a calomel suspension in agar saline. The upper chamber contains mercury and serves to connect with a thin copper wire.

exactly like the potentials previously described for the ureter (4) and they cannot be distinguished from corresponding records of cardiac muscle except for the fact that the duration of the whole complex is five to eight seconds. In agreement with the findings on the other tissues mentioned, the R-T interval is independent of the distance between the leads and, therefore, these two waves do not represent the electric changes at each lead. Increasing the inter-electrode distance merely broadens the R wave and makes the T wave larger as compared with the R wave. With distances of a centimeter or more the waves are so broad that they can no longer be clearly identified. The potentials are usually about twice as large in the pyloric region as compared with the body and are greatest along the greater curvature, but otherwise no differences were noted. In the pause between the complexes, usually lasting eight to ten seconds, no electric activity can be detected even at high amplification.

Not infrequently, particularly with large electrodes, a small downward deflection previous to the R wave was observed. This wave may be considered an artifact which is caused by the action of the organ as a volume conductor and is analogous to similar effects obtained in large muscles. Anti-peristaltic waves were recorded occasionally and gave the same type of potential except opposite in sign.

As previously mentioned, the approximate shape of the monophasic potential can be determined from the records by integration (fig. 3 D, E). It shows a period of negativity maintained at a constant level for a few seconds and is always preceded by a brief hump.

Fig. 2. Differential potentials of two peristaltic waves from the stomach of the dog. Time in seconds.

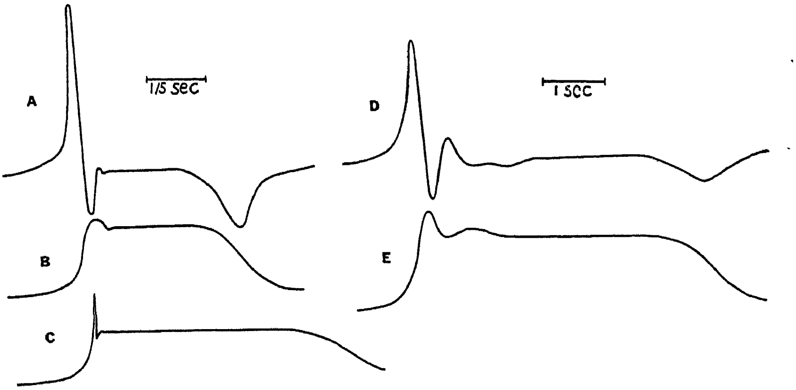

Fig. 3. Derivation of monophasic from differential potentials. A: differential potential from the ureter of the rat (from (4) fig. 2). B: integral curve of A obtained by graphic integration. C: monophasic potential of the rat's ureter recorded by oscillograph. D: differential potential from the dog's stomach. E: integral curve of D, representing the approximate shape of the monophasic potential.

It should be noted that the curve obtained by integration is the only one which conforms with the results obtained on the assumption that the velocity of conduction and other characteristics of the response are uniform in the region of the muscle between the electrodes. As an experimental check on the validity of the method, records from other smooth muscles previously described (4) were analyzed. The results show that the monophasic potential derived from the differential curve agrees with that directly recorded as well as expected (fig. 3 A, B, C). Because of a well-known and inevitable "diphasic artifact" in the usual method of recording monophasic potentials the calculated curve is probably more accurate than the other.

A single electric shock (discharge of a 4μF condenser, 4.5 to 22 volt) applied orad to the leads at the end of a T wave, produced a premature peristaltic wave followed by a prolonged pause (fig. 4 A, B). The pause is sometimes exactly compensatory, but it is more often somewhat shorter. The premature electric response is essentially normal but the R wave is broadened, probably due to slow conduction.

The refractory phase of the muscle is long as shown by the fact that a premature response could not be obtained less than three seconds after the end of the T wave, usually only after five to six seconds. The velocity of conduction of this contraction is 1.5 to 3 mm. per second, the slower the earlier in the cycle the

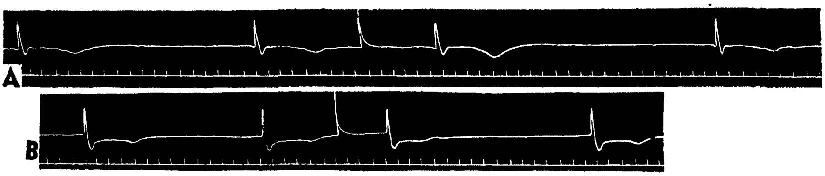

Fig. 4. Premature peristaltic waves in the dog's stomach caused by single shocks orad to the leads. Moment of shock indicated by shock artifact. The pause following the response is compensatory in A, too short to compensate in B. Recorded by mechanical recorder. Time in seconds.

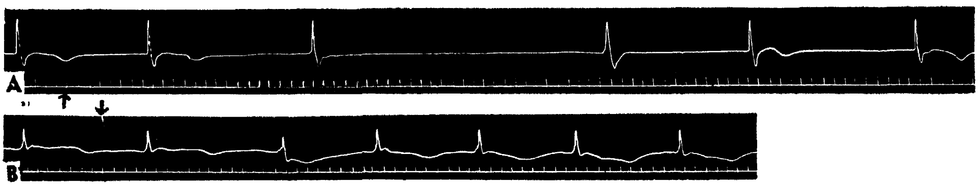

Fig. 5. Effect of intravenous injection of adrenaline on the dog's stomach; 10 gamma per kgm. were injected at the time marked by arrows. In A third complex without T wave, then gradual increase in R-T interval. In B, obtained from a dog less sensitive to adrenaline, the first interval after completion of injection is prolonged, possibly due to slow conduction. Later the intervals are shortened while R-T interval gradually returns to normal. Recorded by mechanical recorder. Time in seconds.

response occurs. In calculating the velocity it has been assumed that the latency of the electric response is negligible, in agreement with experiences on other visceral smooth muscles (6).

In most dogs only very large intravenous injections of adrenaline (0.02 mgm. per kgm.) cause a temporary cessation or slowing of the electric activity of the stomach. Moderate to large amounts of adrenaline actually cause a small increase in the frequency of discharge (fig. 5). At the same time, the R-T interval is reduced, indicating a decrease in the duration of the active phase of each contraction. This effect is similar to that of acetylcholine on auricular muscle.

It is remarkable that adrenaline does not reduce significantly the height of the potentials although it stops all visible movements, a fact also noted by Alvarez (2). It can be stated generally that the height of the potentials is not closely

related to the strength of the contraction as observed visually. The discharge of conducted potentials is as regular as the heart beat for periods of hours and probably is interrupted rarely, if ever, under normal conditions, while the strength of contraction shows marked fluctuations. It would not be justified, however, to conclude that electric and mechanical activity can occur independently. In experiments on intestinal muscle, where similar observations were made, electric and mechanical changes were recorded simultaneously and it was found that every potential was accompanied by a contraction, however weak (6). These observations support the view of Alvarez that peristaltic waves normally originate at a pacemaker in the upper part of the stomach, probably the cardia, even if the waves become readily visible only in the lower portions of the organ.

In the cat the action potentials are like those of the dog as long as the motility of the stomach is very weak. If movements are so strong that they can be easily seen, there are, in addition to the R and T waves, also brief spike potentials which are discharged with great regularity (fig. 6). The number and frequency of these spikes increases with the strength of the contraction. Adrenaline in moderate concentrations stops the spike potentials and shortens the R-T interval. Larger amounts of adrenaline, sufficient to cause a marked rise in blood pressure temporarily cause a complete cessation of all electric activity.

A regular discharge of spikes is recorded only with small electrodes and only if the leads are arranged exactly in the direction of conduction. The irregularities which otherwise are noted can be expected on the assumption that adjacent strands of smooth muscle do not become activated exactly at the same time, perhaps due to slight differences in the velocity of conduction.

In the guinea pig a peculiar difference was noted between the potentials of the pyloric region and other parts of the stomach. In the former, the potentials are similar to those of the cat. The R wave is rather broad but the T wave usually cannot be clearly distinguished because of some irregularities in the graph (fig. 7). In the middle portion of the stomach, on the other hand, the discharge apparently consists only of brief spikes.

The graphs obtained from the guinea pig generally are more irregular than in the other species studied. This is at least partly due to the small width of the peristaltic wave which, as pointed out before, makes it more difficult to resolve the finer details of the electric changes.

Comments. The presence of a sustained state of negativity is the most general feature of the potentials of the stomach and in this respect the results agree closely with those obtained for other visceral smooth muscles, the ureter and the intestine (6). In the cat, however, brief spikes are superimposed on the sustained potential and in the guinea pig the spikes may be the only conspicuous part of the potential. Similar species differences were found in the ureter and, in every case, the guinea pig showed the greatest predominance of spike potentials.

The significance of the spike potentials is unknown. For the ureter of the dog, cat and rat it has been shown that either a potential with a smooth plateau or with a regular repetitive discharge of spikes can be obtained depending on the composition of the Ringer's solution used (6), while at the same time the mechan-

ical responses are essentially the same. It appears, therefore, that the various types of potentials, however different they may be, are physiologically equivalent. This is not surprising if we assume that the energy release of muscle continues during the whole period of negativity. Physiologically, then, it is not very significant whether the potential change is a rapid series of brief spikes, as in a true tetanus, or a prolonged period of negativity as commonly found in visceral muscles. In as much as the active phase of the contraction is prolonged, the responses of visceral muscles, as previously suggested, may be considered fundamentally as tetanic contractions even if spikes are absent.

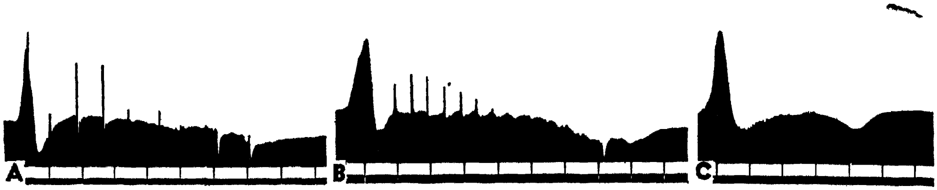

Fig. 6. Potentials from the stomach of the cat. A and B: differential potentials associated with one peristaltic wave of moderate strength. C: after intravenous injection of adrenaline (3 gamma per kgm.). Time in seconds.

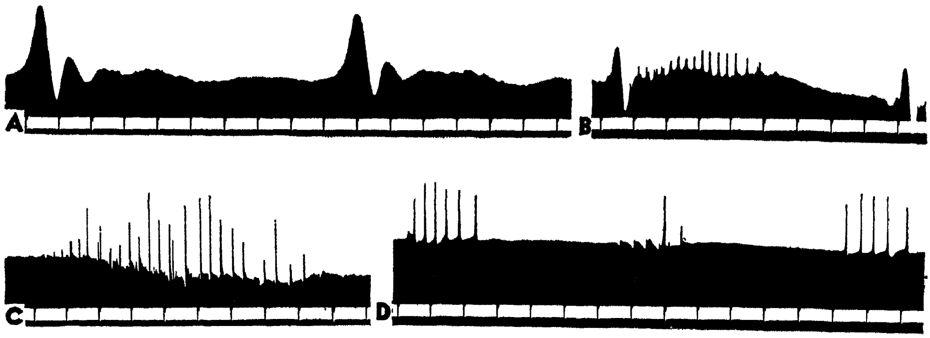

Fig. 7. Potentials from the guinea pig's stomach. A and B from pyloric region. C and D from middle portion of organ. C: single strong contraction. D: weak contractions. Time in seconds.

On the basis of these findings the concept of the impulse is not readily applicable to smooth muscle if the action potential is used as the chief criterion. The term is useful in describing the activity of cardiac muscle and of nerve fibers. It is an all-or-none response which makes up all the conducted responses of these tissues and is characterized by an action potential which is either a brief spike or, as in cardiac muscle, a period of sustained negativity. In visceral smooth muscle, on the other hand, an all-or-none response to a single threshold stimulus, even in the same muscle, may show either a single spike, a series of spikes, a plateau, or a combination of these elements.

The similarity of the action potentials of the stomach and of other visceral muscles with those of the heart suggests that the mechanism of conduction is fundamentally the same in all of these muscles and this conclusion is supported

by numerous other considerations. A nervous structure which might have the function of conduction in smooth muscle has not been demonstrated histologically (8). More important, observations on isolated muscle cannot be reconciled with the assumption of nervous conduction (3). The most direct studies on the question of conduction are those on the effect of an electric current on visceral muscles. They show that no electric polarization develops at the boundaries of the muscle fibers. Consequently no barrier is present for the conduction of impulses from cell to cell or, in other words, the muscles behave like syncytia.

As has been pointed out previously (5), also the injury potentials and action potentials of visceral muscles can be explained only on the assumption that these muscles are syncytia. For instance, if smooth muscle consisted of independent fibers, like skeletal muscle, a wave of contraction, whatever the mechanism of conduction, could not produce a monophasic potential if one lead is on an inactive portion of a muscle. As seen histologically, the fibers of smooth muscle are overlapping and exceedingly short. Even the smallest electrode used leads off from thousands of fibers. The inactive fibers in the injured region would merely act as an extension of the lead and the activity of each remaining active fiber between and under the leads would be recorded as a diphasic potential. It is difficult to see how by the summation of numerous asynchronous diphasic potentials a state of negativity could be maintained for several seconds at one lead. The correctness of this argument is well demonstrated by Adrian (1) in experiments on certain skeletal muscles in which the fibers, although many times larger, have an overlapping arrangement like that of smooth muscle.

A similar consideration applies also to the diphasic potential. If both leads are on active tissue each unit between the leads produces a diphasic potential. Since, during conduction, the units will be activated successively a polyphasic electric response should be recorded if a small number of units is present. In a system with units as small as smooth muscle fibers the diphasic potentials, being asynchronous, would almost completely cancel out. Actually the potential produced by the stomach of the dog or cat usually is as high as 5 millivolts if the leads are one millimeter apart, as large or larger than those of the turtle heart under the same conditions.

SUMMARY

The potentials of the dog, cat and guinea pig stomach were recorded by means of non-polarizable differential electrodes. The approximate shape of the monophasic potential can be derived mathematically from the records obtained.

In the dog, the differential potential associated with each peristaltic contraction shows three main waves which are designated as R, S and T waves. The shape of the potential is identical with that of some other visceral muscles and of cardiac muscle but the complex lasts for five to eight seconds. During the intervals between each complex there is a period, lasting about eight seconds, of complete rest. The monophasic potential derived from the differential curves shows a sustained negativity lasting for several seconds.

A single shock applied a few seconds after a T wave produces a premature peristaltic wave. It is followed by a prolonged pause which is often compensatory.

Adrenaline shortens the R-T interval but it stops electric activity only in very high doses.

In the cat the same potential as in the dog is obtained as long as the contractions of the stomach are weak. During moderate and strong peristalsis, brief spikes are superimposed on the slow phases. In the guinea pig a slow potential can be observed only in the region of the pylorus, whereas in the middle portion of the stomach the discharge consists only of brief spikes. These differences between species are similar to those previously found in other visceral muscles.

It is shown that the potentials observed in visceral smooth muscles cannot be explained on the assumption that the muscle fibers are independent units. The results are in agreement with the conclusion derived from other observations that conduction is due to a syncytial arrangement of the muscle fibers.

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THE EFFECT OF THIOCYANATE ON GASTRIC POTENTIAL AND SECRETION

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Previous studies have demonstrated that under certain experimental conditions there is a relationship between the potential difference across the stomach wall and the secretion of HCl (6, 7). It was found that histamine stimulation resulted in a decrease in the magnitude of the potential. The potential of the resting stomach was in the majority of the experiments between 70 and 80 millivolts and after histamine stimulation it decreased to approximately 40 millivolts. Certain agents were found to decrease the potential (6, 7) of the resting stomach without stimulating the stomach to secrete HCl. Histamine administration during the period in which the potential of the resting stomach was depressed was followed by relatively little decrease in the potential and a normal secretory response. The level of the potential of the secreting stomach, however, was the same (around 40 millivolts) irrespective of the level of the potential before histamine stimulation. These same agents were found at times to decrease the potential of the secreting stomach and this decrease was associated with a concomitant fall in the secretory rate.

Several investigators (1, 2) have reported that the administration of thiocyanate depresses the ability of the stomach to secrete HCl. In an attempt to elucidate further the relationship between the gastric potential and the secretion of HCl the following studies were made on the effect of thiocyanate on the gastric potential and secretion. A preliminary report of these investigations appeared elsewhere (4).

METHODS. The technique for measuring gastric secretion and potential, described in a previous paper (6), was employed. With this technique a portion of the stomach along the greater curvature is placed between a ring of lucite and a lucite chamber. The ring of lucite has an oblique cut in it so that the blood vessels could be slipped inside it, thereby insuring an intact blood supply to the portion of the stomach in the chamber. The mucosal side of the stomach was oriented toward the chamber and the chamber was filled with normal saline. The secretory rate was determined every ten minutes by draining and flushing the chamber with saline. The pH and titratable acidity were determined on each sample in most of the experiments. In some experiments only the pH was measured. A non-polarizable electrode made contact with the fluid in the chamber and another similar electrode was placed in contact with the serosa. The potential difference was measured with a potentiometer.

Dogs were used in these experiments and in the majority of experiments pernoston (80 mgm. per kgm. subcutaneously) was used as the anesthetic agent.

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In a few experiments sodium amytal was used (90 mgm. per kgm. subcutaneously). The results with the two anesthetics were essentially the same. Blood pressure was recorded in the usual way with a U manometer.

RESULTS. *Effect of thiocyanate on potential and secretion in the secreting stomach.* In a total of nine dogs histamine (0.02 to 0.1 mgm. per kgm. of the diphosphate) was injected subcutaneously at regular intervals and after secretion was established sodium thiocyanate was injected intravenously. Figure 1 represents a typical experiment in which the thiocyanate was administered in divided doses. The first histamine injection was made at the time indicated by the arrow marked H, and repeated at ten minute intervals for the duration of the experiment. At the times indicated by the arrows labeled T the indicated amounts of sodium

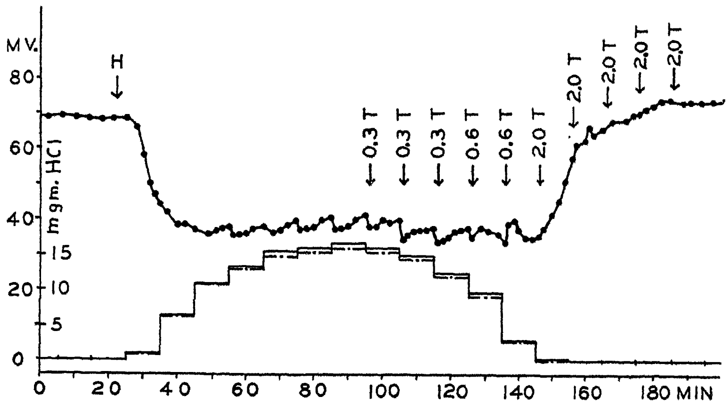

Fig. 1. Effect of thiocyanate on secretion and potential of stomach stimulated with histamine. At arrow marked H, 1 mgm. histamine diphosphate was injected subcutaneously. At ten minute intervals after the first injection 0.5 mgm. of histamine diphosphate was administered for the duration of the experiment. Arrows labeled T indicate times at which sodium thiocyanate was injected intravenously. The amounts in grams are given in the figure. The solid dots represent the potential difference across the stomach wall in millivolts. The straight lines (obtained from titration data) and the dash-dot lines (obtained from pH data) represent the rate of secretion of HCl in milligrams per ten minute periods. Weight of dog, 17.0 kgm. Sodium amytal anesthesia.

thiocyanate were injected intravenously. It can be seen that, after the injection of a total of 4.1 grams of thiocyanate (after the sixth injection), the potential showed a marked increase and reached a level of approximately the same magnitude as that of the resting stomach. It can also be seen that the secretory rate showed a steady decrease reaching a value of approximately 20 per cent of its value previous to the first injection of thiocyanate before the potential started its upward climb. This decrease in secretory rate, before the increase in potential, was found in those experiments in which the thiocyanate was administered in the above manner.

On the other hand, as can be seen in figure 2A, the administration of a relatively large amount of thiocyanate on the first injection was followed by an immediate increase in the potential and a drop in the secretory rate during the following ten minute period to a very low value.

In some experiments, as illustrated in figure 2B, a single injection of thiocyanate may produce a temporary increase in the potential. After the return of the potential to its pre-injection value it can be seen that the secretory rate does not return to its pre-injection level but remains at a very low level.

In the above experiments the average level of the potential before histamine stimulation was 75 mv. (S.E. ±1.7 mv.). After the potential had reached a relatively constant value following histamine stimulation it was 39 mv. (S.E. ±1.1 mv.). The average level following the increase in the potential resulting from thiocyanate injection was 73 mv. (S.E. ±2.1 mv.).

After the increase of the potential to the level of the non-secreting stomach following thiocyanate injection an increase in the amount of histamine may result in the reinitiation of secretion which again is accompanied by a lowering of the potential.

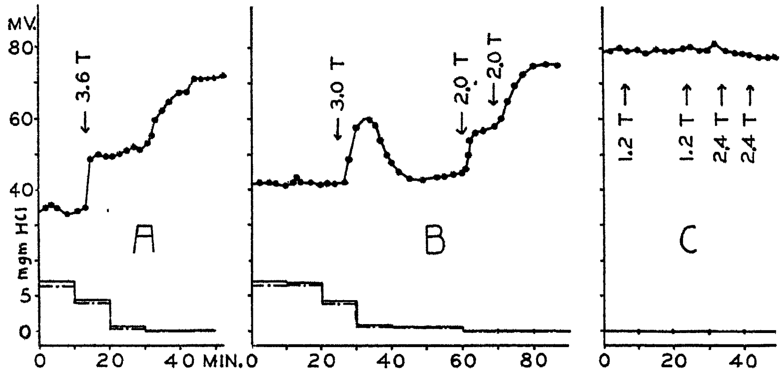

Fig. 2. A and B. Effect of thiocyanate on potential and secretory rate of secreting stomach. Weight of dog in A, 16.3 kgm.; that in B, 18.4 kgm.

C. Effect of thiocyanate on potential of resting stomach. Weight of dog 17.7 kgm. The potential one hour after the last reading shown in the figure was 79 mv.

A, B and C. Conventions same as in figure 1. Ordinates on left apply to all three parts of figure. Abscissae represent time in minutes. Pernoston anesthesia in all three dogs.

In control experiments it was found that the secretory rate could be maintained for long periods of time with the doses of histamine employed in the above experiments. The level of the potential remained remarkably constant until the death of the animal, following which the secretion of HCl ceased and the potential dropped to approximately zero.

Effect of thiocyanate on the potential of the non-secreting stomach. It is possible that the increase in the potential following thiocyanate injection is not dependent on the secretory state of the stomach and that the administration of this substance to the non-secreting stomach would result in an increase in the potential. Four experiments were performed in which thiocyanate was administered to the non-secreting stomach in amounts larger than those required to produce an increase in the potential of the secreting stomach. Figure 2C represents a typical experiment, and it can be seen that thiocyanate does not produce a definite change in the potential of the non-secreting stomach. Continued administration

of large quantities of thiocyanate eventually resulted in the death of the animal, which was accompanied by a fall of the potential to a value very close to zero.

Effect of thiocyanate on blood pressure. In preliminary experiments it was found that rapid injection of the larger doses of thiocyanate often resulted in a marked decrease in pulse rate which was associated with a marked fall in blood pressure. Under these conditions there was a decrease in the potential in both the secreting and non-secreting stomach. The decrease in the potential of the secreting stomach was associated with a decrease in secretory rate. In the experiments reported above the thiocyanate was injected over a period of approximately one to three minutes depending on the dose. At this rate of injection and with the doses employed the marked cardiac inhibition was absent, and, apart from an occasional small temporary decrease in blood pressure, the thiocyanate had either no effect or caused a rise in blood pressure. The blood pressure showed an increase in some experiments of over 50 per cent and gradually returned toward its previous level over a period of ten or more minutes. Injection of thiocyanate at ten minute intervals, or a single injection of a large dose, was accompanied usually by a sustained increase in blood pressure for over an hour or more. These results on the effect of thiocyanate on blood pressure in acute experiments are quite similar to those reported by Nichols (3).

Discussion. The above experiments present further evidence that a relationship exists between the secretion of HCl and the gastric potential. It has been pointed out before (6) and also illustrated in figure 1 of this paper that, under the above described experimental conditions, the potential after histamine stimulation reaches a relatively constant level while the secretory rate may continue to increase for some time. It is interesting to note in this connection that thiocyanate may produce a marked fall in secretory rate before the potential shows a definite increase. The secretory rate may therefore show wide changes, either an increase or a decrease, while the potential of the secreting stomach as measured by the present technique shows relatively little change. However, it should be pointed out that the potential difference measured with the present method is undoubtedly influenced by the diffusion potential originating at the junction between the gastric juice and the saline in the chamber. For a study of the relationship between the magnitude of the gastric potential minus this diffusion potential and the secretory rate a previous paper should be consulted (6). On the basis of the present experiments and those reported before (6, 7) it appears that once the secretory rate has reached a relatively constant value either a marked increase or decrease of the potential is associated with a marked decrease in secretory rate.

In a series of experiments (5–7) including those presented in this paper an attempt is being made to find out whether electrical energy furnishes the energy necessary for the production of HCl by the stomach. One avenue of approach to this problem is the study of the relationship between the gastric potential and the rate of secretion of HCl. It would obviously be much more desirable to be able to study the relationship between the rate of electrical energy production and the rate of HCl production. However, it has not been possible as yet to

determine the electrical energy output of the stomach apart from the minimum amount occurring when the potential is shunted through an external conductor (5). Assuming the presence of shunts across the electromotive forces of the stomach, the potential difference across the stomach wall would be a function of both the magnitudes of the electromotive forces and the resistance of the various parts of the electrical circuit of the stomach. A decrease in the potential might be associated with an increase in the electrical energy production and vice versa. It is also possible that the electrical energy production could increase or decrease without a change in the potential (the electromotive force and resistance changing in opposite directions). It should be kept in mind, therefore, that the potential difference is not a measure of electrical energy output any more than mean arterial pressure is a measure of cardiac output.

SUMMARY

Intravenous administration of sodium thiocyanate in appropriate amounts results in complete inhibition of the secretion of HCl by the stomach and an increase of the potential difference across the stomach to the resting level. Administration of thiocyanate in smaller amounts results in an inhibition of the secretory rate before the potential increases. Thiocyanate in comparable amounts has no demonstrable effect on the potential of the resting stomach. The relation between these findings and previous ones is discussed.

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STREAMLINE FLOW IN THE ARTERIES OF THE DOG AND CAT

Implications for the Work of the Heart and the Kinetic Energy of Blood Flow

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Hess (1) has described a series of observations going to show that blood flow in the aortae of various mammals is streamlined (viscous, laminar) in nature. Using a stethoscope applied over the artery at various points, he was unable to hear murmurs, which he interpreted to mean that no turbulence was present. In a later experiment he forced a viscous solution colored with methylene blue into the aorta by way of the carotid artery, and observed blue filaments, distinct from the rest of the blood as it emerged from the cut femoral artery, again indicating streamline flow.

Hahn, Donald and Grier (2) have brought forward strong evidence for streamline flow in the portal vein of the dog. They injected radioactive phosphorus into the splenic and mesenteric veins, and found that the phosphorus was differentially concentrated in the left and right side of the liver, depending upon the vein injected. This was not the case when the injection was made into the jugular vein.

Method. We have modified Hess' experimental technique by inserting a thin-walled glass tube, 2.6 cm. long and 0.2 cm. internal diameter, into the arteries of heparinized dogs and cats. The tube was brightly illuminated from both sides, and observed through a dissecting microscope. India ink was then gently introduced into the left ventricle of the heart or into the descending thoracic aorta through a no. 27 needle, oriented in the direction of the movement of blood. This technique is essentially that used by the British engineer Reynolds (3) in his classical researches on laminar and turbulent flow.

Results and discussion. When ink is introduced as described into the left ventricle or into the aorta, over a period of several cardiac cycles, well-defined streamline filaments of ink may be seen in the glass tube inserted into the lower abdominal aorta or into the femoral artery. This suggests that the flow of blood is streamlined not merely in the arteries, but also at the aortic orifice. We say "suggests" because, to be perfectly rigorous, one would need to visualize the flow pattern at every point along the path of blood flow. To satisfy ourselves on this point we have performed experiments with a simple glass model carrying water. The system included a segment so constructed that it produced marked turbulence, whereas the flow above and below the segment was streamlined. When ink was introduced into the system at a point well above the region of turbulence, the ink was observed to form a new streamline pattern at a distance below the point of turbulence equal to about 50 times the tube diameter.

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This pattern consisted of a very broad band having a parabolic front. Conceivably, such a band might assume the appearance of a sharp filament at a much greater distance from the point of turbulence. Consideration of the concentration of ink necessary to produce such well defined filaments as we have observed in the arteries, however, makes it very unlikely that turbulence had occurred at the aortic orifice or in the aorta. Our experiments, taken in conjunction with the failure of Hess to hear murmurs at any part of the aorta, indicate beyond reasonable doubt the streamline nature of the blood flow both at the aortic orifice and in the arteries studied.

We have made a few observations of the blood flow in the femoral vein and inferior vena cava of the cat which show the streamline nature of the flow in these vessels. If these veins, which are fairly transparent, are compressed to a suitable degree while they are being observed under a good light, the blood may be observed to be thrown into violent eddies, characteristic of turbulent flow, central to the point of compression. Upon release of the vessel, all signs of eddies promptly disappear, and momentary streamline filaments of blood may be seen. Such observations support the findings of Hahn et al., mentioned above.

Admitting the viscous nature of the blood flow at the aortic orifice and in the great arteries and veins, it follows that the customary evaluation of the kinetic energy term in the formula for the work of the heart, and of the kinetic energy of blood flow in the vessels, requires revision. The following mathematical analysis is adapted from Bingham (4) and Vennard (5).

Consider a tube of radius R and length L through which a fluid of viscosity n is flowing under an effective pressure P. Let the radius of a small hollow cylinder coaxial with the tube be r. We may then relate the velocity of the fluid v to r as follows: the tangential force due to the viscous resistance is equal to $2\pi rLn\,\frac{dv}{dr}$, and for steady flow this is equal to the driving force, $-\pi r^2 P$. Solving for dv, we get:

$$dv = -\frac{P}{2Ln}\,r\,dr,$$

which yields on integration,

$$v = -\frac{Pr^2}{4Ln} + K,$$

where K is the integration constant. Since $v = 0$ when $r = R$,

$$K = \frac{PR^2}{4Ln},$$

and therefore

$$v = \frac{P}{4Ln}\,(R^2 - r^2) \tag{1}$$

This is the equation of a parabola, and constitutes the theoretical basis for the statement that the velocity profile of a fluid in viscous flow is a parabola. The parabolic nature of the flow pattern is clearly visible experimentally.

The kinetic energy of the fluid passing any cross-section of the tube *per unit time* is:

$$K.E. = \int_0^R \tfrac{1}{2}\cdot 2\pi r\, dr\, v\rho v^2 = \int_0^R \pi\rho v^3 r\, dr$$

where ρ is the density of the fluid.

Substituting the value of v from equation (1) we get:

$$K.E. = \frac{\pi\rho P^3}{64L^3 n^3}\int_0^R (R^2 - r^2)^3 r\, dr = \frac{\pi\rho P^3 R^8}{512L^3 n^3}$$

$$= \pi\rho R^2\left(\frac{PR^2}{8nL}\right)^3. \qquad (2)$$

From Poiseuille's Law:

$$q = \frac{\pi PR^4}{8nL}$$

we get:

$$I = \frac{q}{\pi R^2} = \frac{PR^2}{8nL} \qquad (3)$$

where I is the *mean velocity* and q is the volume flow per unit time.

Hence we can write equation (2) as:

$$K.E. = \pi\rho R^2 I^3 \qquad (4)$$

Turning now to Bernoulli's theorem for the flow of an *ideal* fluid, which holds, practically, for turbulent flow,

$$PV + \tfrac{1}{2}mv^2 + mgh = \text{constant},$$

where V is volume, m is mass, g is the acceleration due to gravity, and h is the height of the fluid above a given datum plane. The term mgh is the "energy of position," and will be omitted from later equations. The kinetic energy *per unit time* may be written:

$$K.E. = \tfrac{1}{2}\pi R^2\rho I\cdot I^2 = \tfrac{1}{2}\pi\rho R^2 I^3 \qquad (5)$$

I may be substituted for v because the velocity profile is a straight line, and this is also nearly true for turbulent flow.

Comparing equations (4) and (5), we see that the kinetic energy term in viscous flow is *twice as great* as that in ideal flow. Another way of saying this is to write Bernoulli's theorem:

$$PV + \alpha\cdot\tfrac{1}{2}mI^2 = \text{constant}.$$

The value of α depends upon the nature of the flow, being 2 for viscous flow, and 1 for ideal flow. In turbulent flow, α has a value close to unity, 1.05 being frequently used by hydraulic engineers.

Turning now to the work of the heart, we wish to emphasize that earlier formulae *implicitly assume an ideal type of flow.* Thus Evans (6) writes the *K.E.* term $\frac{1}{2}mI^2$, while Katz (7) uses the more refined integral expression $\int \frac{1}{2}I^2\, dm$. (We are working with absolute units, hence the omission from these formulae of the acceleration due to gravity, g.) For viscous flow, however, the factor $\frac{1}{2}$ should be omitted. Thus the usual approximate formula for the work of the heart should be written:

$$\text{Work of left ventricle} = PV + mI^2,$$

where P is the mean aortic pressure, V is the stroke volume, m is the mass of blood ejected, equal to ρV, and I is the mean velocity, equal to the rate of volume flow divided by the cross-sectional area of the aortic orifice.

Similarly, the kinetic energy is written mI^2 wherever the flow is streamlined.

So far as the work of the heart and the kinetic energy of flow in the arteries are concerned, the value of the kinetic energy term is still small compared with the pressure term, so long as relatively low velocities of flow occur. Thus, taking approximate values of P, V, m and I for the human left ventricle under resting conditions, the kinetic energy term turns out to be of the order of magnitude of 1.8×10^5 ergs (183 grams-cm.) per stroke, while the pressure term is of the order of magnitude of 8×10^6 ergs (8,200 grams-cm.) per stroke. Katz (ibid) working with the isolated ventricle of the turtle, in one case obtained a value for the kinetic energy of 359.5 grams-cm., which seems impossibly high, even though the very approximate nature of the simple formula we have used above is admitted. We are of the opinion that some of the findings of Katz concerning the relative rôles played by velocity energy and pressure energy in the work of the heart are open to very serious doubt, or else that the turtle ventricle is grossly different in its mechanical behavior from the mammalian ventricle.

It should be noticed that the classical formula should be used when the flow is known to be turbulent, as perhaps may occur in arduous muscular exercise, anemia, and various cardiovascular states characterized by the presence of murmurs.

SUMMARY

1. Blood flow in the aorta and femoral artery of the cat and the femoral artery of the dog has been studied by inserting a short glass tube into the arteries and observing the flow pattern of injected India ink.

2. The streamlined nature of the flow is evident when the ink is injected as described either into the left ventricle or into the aorta.

3. Some observations on blood flow in the femoral vein and inferior vena cava of the cat are described, showing the streamlined nature of the flow in those vessels.

4. Mathematical analysis shows that the kinetic energy term in streamlined flow is twice that in ideal flow. The formulae for the work of the heart and the kinetic energy of blood flow wherever the flow is streamlined are accordingly modified.

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HISTAMINE-LIKE SUBSTANCE PRESENT IN NASAL SECRETIONS OF COMMON COLD AND ALLERGIC RHINITIS[1,2]

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The presence of a histamine-like substance in the nasal secretions of allergic rhinitis was investigated by Buhrmester and Wenner (1) in 1936. Some extracts which they obtained produced a fall in blood pressure when injected into the anesthetized, atropinized male cat; they concluded, however, that the small amount of depressor activity obtained was probably due to proteoses which had formed in their extracts during hydrolysis. No evidence of the presence of histaminase could be found in nasal secretions.

In this paper, the total amount of histamine extractable from nasal secretions by the method herein outlined has been investigated. Secretions obtained both from cases of allergic rhinitis and "common cold" were studied. A histamine-like substance, the total concentration of which varied widely, was detected in most samples of both types of nasal secretion; time did not permit a further investigation as to the factors influencing the wide variations in total histamine content.

METHODS. *Collection and extraction of samples.* The method of obtaining an extract of "histamine" from nasal secretions, suitable for assay on strips of guinea-pig ileum, was based on procedures used in extracting histamine from blood by Barsoum and Gaddum (2), Code (3), Anrep, Barsoum, Talaat and Wieninger (4), Emmelin, Kahlson and Wicksell (5) and Kwiatkowski (6). Nasal secretion was collected by aspiration or by means of cellophane sheets, into which the patients were instructed to blow their nose. The secretions were immediately transferred with a syringe into a weighed centrifuge tube containing 1 cc. of 10 per cent trichloracetic acid. The weight of nasal secretion obtained was determined by difference; usually at least one gram of secretion was collected from each patient. If a greater amount was secured, 10 per cent trichloracetic acid was added to make its total volume roughly equal to that of the nasal secretion. A history of each patient was always taken; when possible, a smear of the nasal secretions was made in order to estimate the eosinophile content.

After standing overnight in the refrigerator, the sample was centrifuged and the supernatant liquid transferred by means of a syringe and needle to a micro-funnel fitted with a hardened filter paper. Both centrifuging and filtration were necessary to facilitate complete removal of the trichloracetic acid precipitate. Three 1 cc. portions of 10 per cent trichloracetic acid were used to wash the pre-

[1] Aided by a grant from the Christine Breon Fund, University of California Medical School.

[2] This report covers one phase of joint studies on the physiologic mechanisms concerned with the etiology of the common cold.

cipitate. To the combined filtrates an equal volume of concentrated HCl was added and the mixture refluxed for 90 minutes. It was evaporated almost to dryness at about 50°C. by means of a stream of nitrogen. Four 10 cc. portions of absolute alcohol were added and in turn similarly evaporated from the sample, which was finally completely dried. The residue was extracted four times with (2 + 1 + 1 + 1) cc. of absolute alcohol previously saturated with NaCl. The combined alcoholic filtrates were evaporated to dryness at 40° to 50°C. and stored in a refrigerator for assay on a strip of guinea-pig ileum. These dried alcoholic extracts were taken up in Tyrode's solution (usually 1 cc.) on the day of assay. The pH was adjusted with $\frac{N}{5}$ NaOH and phenol red indicator. Blank determinations, made by dissolving the residue from 5 cc. of the absolute alcohol saturated with NaCl in 1 cc. of Tyrode's solution produced no effect on guinea-pig ileum.

Assay on guinea-pig ileum. A very sensitive method of assay was needed, since it was anticipated that it would be difficult to obtain large samples of nasal secretions and that the quantity of histamine present might not be great. The technic described by Kwiatkowski (6), which is based on the principles used by Gaddum, Jang and Kwiatkowski (7) for the detection of small amounts of sympathin, was adopted and used to obtain all the results shown in table 1. In this method, an arrangement is set up instead of the usual bath whereby the Tyrode's solution drops at a constant rate from a capillary tube onto the strip of guinea-pig ileum suspended in a small, moist chamber. A dose of 0.1 cc. of the solution to be tested is injected into an arm of the capillary tube and subsequently falls undiluted onto the muscle strip. It was felt that the results obtained in this manner were satisfactory; however, a few determinations (table 2) were carried out in a conventional bath of oxygenated Tyrode's solution, in which the strip of ileum was immersed.

Tyrode's solution was made up according to Burn (8), except that the concentration of $CaCl_2$ was halved (6); atropine sulfate was added as indicated in the tables. A constant temperature of 36°C. was maintained in all assays.

Calculations. Whenever possible, the biological assay was conducted according to the design set forth by Schild (9), and the results statistically analyzed as outlined by this author. However, since the quantity of unknown solution was definitely limited, the results obtained were sometimes not suitable for statistical analysis; in such cases a fair approximation could be made.

Results. In table 1 the results obtained by the perfused strip technic of Kwiatkowski (6) are outlined. The first eight determinations listed were done on nasal secretions obtained from patients having a "common cold." The next nine determinations were made on nasal secretions produced by an allergic condition. In each group wide variations in histamine-like activity were observed; the range of variation was essentially the same in both groups with the exception of U32, which was a very purulent sample. U16 and U20 were samples taken from the same patient. There appears to be no striking difference in total histamine-like activity between secretions of "common cold" and those of allergic

origin. A comparison of the activity of U11 with U12 is interesting, however; these samples were taken from the same patient on the same day. A typical

TABLE 1

Extracts of nasal secretion assayed for histamine-like activity by perfusion of guinea-pig ileum in vitro

SAMPLE		NASAL SECRETION OBTAINED	EOSINOPHILE CONTENT	ESTIMATED HISTAMINE-LIKE ACTIVITY (AS HISTAMINE DIPHOSPHATE)	LIMITS OF ERROR* OF ESTIMATE
		grams		*µg./gm*	*µg./gm.*
1. U1	Common cold	1.01		>0.1	
2. U2	Common cold	0.48		>0.1	
3. U7	Common cold	0.70	3+	0.09	0.07–0.12
4. U8	Common cold	0.80	1+	0.21	0.15–0.28
5. U11	Common cold	0.45		0.10 (maximum)	
6. U22	Common cold	3.14		0.046	0.030–0.069
7. U23	Common cold	3.30		Not detectable†	
8. U32	Common cold	1.50		1.6‡	0.8–2.0
9. U12§	Common cold plus allergic rhinitis	1.40	4+	0.18	0.14–0.23
10. U13	Allergic rhinitis	1.84		0.03	
11. U14	Hay fever	0.38		Present (<0.07)	
12. U15	Hay fever	0.99	2+	0.028	0.014–0.056
13. U16	Hay fever	0.82	3+	0.064	0.047–0.086
14. U17	Hay fever	2.35	2+	0.0091	0.007–0.012
15. U20	Hay fever	2.02	1+	0.059	0.035–0.10
16. U18 U19	Allergic rhinitis Hay fever	2.33		0.24	0.16–0.38
17. U24	Common cold plus allergic rhinitis	2.71		Present (<0.04)¶	
18. U25 U26 U27 U28	Allergic rhinitis 8.72 Common cold 2.35 Common cold 1.29 Common cold 1.50	13.86	1+	0.018**	0.012–0.026

* P. 0.99 limits of error. Where limits of error are not given, data were insufficient for statistical analysis according to Schild (9) and the estimated activity can be regarded only as an approximation.

† 0.06 µg./cc. was the limit of sensitivity in this assay. Atropine sulfate, 500 µg./liter, used in Tyrode's solution.

‡ Atropine sulfate 500 µg./liter. Purulent nasal secretion. Amount of precipitate obtained with trichloracetic acid was very large.

§ Sample taken from same patient and on same day as U11, after exposure to rabbits and guinea pigs.

¶ Atropine sulfate 500 µg./liter.

** Atropine sulfate 500 µg./liter added to Tyrode's solution for last half of assay; no significant change in responses produced by unknown relative to standard was noted.

"common cold" occasioned the taking of the first sample (U11). While this was being obtained, the history of the patient revealed a susceptibility to allergic rhinitis caused by guinea pigs and rabbits. After U11 was obtained, the patient

consented to visit the room where our experimental animals are kept. Within a few minutes he began to sneeze and the flow of nasal secretion was considerably increased. U12 was collected after the attack of allergic rhinitis was established. As is shown in table 1, the concentration of histamine-like activity in U12 was very definitely greater than in U11. It is also interesting that the eosinophile content of U12 was higher than that of U11.

As indicated in table 1, atropine sulfate was only used in a few assays [U23, U32, U24, and U(25 + 26 + 27 + 28)]. We found, in agreement with Kwiatkowski (6) and Code (3), that the addition of atropine sulfate (0.1–1.0 μg./cc.) may reduce the response of the ileum to histamine sufficiently to cause difficulty in the estimation of weak extracts. However, the activity determined in the

TABLE 2

Extracts of nasal secretion assayed for histamine-like activity on guinea-pig ileum suspended in bath

SAMPLE	NASAL SECRETION OBTAINED	EOSINOPHILE CONTENT	ESTIMATED HISTAMINE-LIKE ACTIVITY (AS HISTAMINE DIPHOSPHATE)
	grams		*μg./cc.*
1. U30 "Common cold"........	5.5		0.28–0.55
2. U31 Same patient as U30, one day later........	7.0		0.20
3. U35 Hay fever	2.7	2+	Not detectable (<0.55)*
4. U36 Same patient as U35. Hay fever, one week later. . .	2.7	2+	Present (<0.20)†
5. U33 Common cold 3.2; U37 Hay fever 4.3; U38 Hay fever 2.3; U39 Common cold 3.0 }	12.8	3+ 3+	0.4‡

* 0.55 μg./cc. of secretion was the limit of sensitivity in this assay. Atropine sulfate, 100 μg./liter, used in Tyrode's solution.

† Atropine sulfate, 100 μg./liter, used in Tyrode's solution.

‡ Atropine sulfate, 100 μg./liter, had been in muscle bath for 5 minutes, after which muscle strip was washed just previous to assay. See figure 1.

assays already indicated in table 1 as well as in U35, U36 and possibly U(33 + 37 + 38 + 39) of table 2, was atropine resistant. In the determination of U(25 + 26 + 27 + 28), atropine sulfate was added to the Tyrode's solution during the last half of the assay; no significant change in the responses produced by the unknown relative to the histamine standard was observed. It would seem that at least a large part of the smooth muscle-stimulating activity present in our extracts was atropine resistant.

The statistical calculations carried out according to Schild (9) always gave a highly significant F value for regression between the smaller and larger dose of standard and unknown. The deviations from parallelism (9) between standard and unknown were always insignificant; therefore, the active substance in the extracts of nasal secretion appeared to have a dose-response curve similar to that of histamine.

The extracts of samples listed in table 2 were assayed by means of a strip of

guinea-pig ileum immersed in an ordinary 15 cc. bath of oxygenated Tyrode's solution, instead of the perfusion method of Kwiatkowski (6) used in table 1. It was felt that it would be desirable thus to check the results obtained by the perfusion method. The bath method is much less sensitive than the perfusion method. However, only one extract was actually negative (U35), and a second

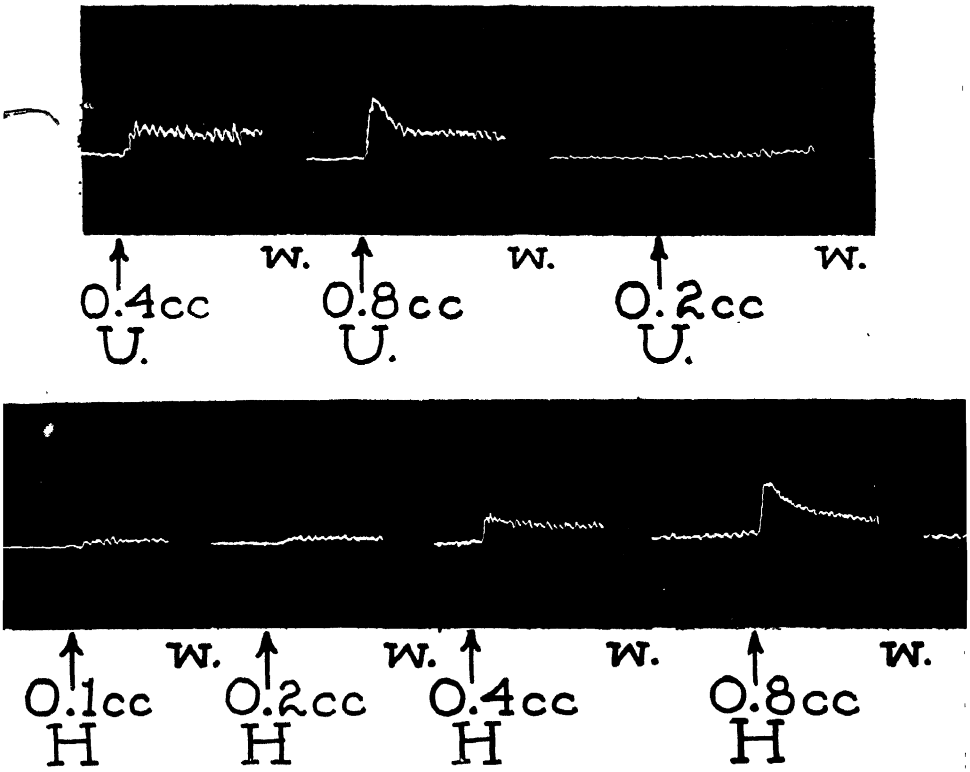

Fig. 1. Comparison of U(33 + 37 + 38 + 39) with histamine acid phosphate on guinea-pig ileum suspended in a 15 cc. bath of oxygenated Tyrode's solution. Temp. 36°C. Bath had contained 100 μg./liter atropine sulfate for 5 minutes, after which ileum was washed with Tyrode's solution just previous to this assay.

H—Histamine acid phosphate, 2.76 μg./cc., (1 μg./cc. histamine base).

U—Extract of 12.8 cc. nasal secretion in 2 cc. final volume.

W—Washout and rest (5 min.).

Experiment was continuous throughout the seven doses.

sample from the same patient did show some activity. The record of activity of U(33 + 37 + 38 + 39) is portrayed in figure 1. No correlation was apparent between the number of eosinophiles and the amount of histamine present in the nasal secretion.

Comment. The possible significance of histamine in anaphylaxis and allergy has been adequately reviewed (10–15); however, histamine has not, to our knowledge, previously been linked with the common cold. The failure of Buhrmester and Wenner (1) to detect definite histamine-like activity in their extracts of allergic nasal secretion can be attributed to the inadequacy of their method.

It can not be stated with absolute certainty that the active substance in our extracts is histamine. Our method of extraction is based on principles used by others (2–5) in extracting histamine from blood for assay on atropinized guinea-pig ileum; presumably the only active substance present was histamine. Inhibiting substances and a "slow reacting substance" (2) were destroyed by boiling in HCl.

The smooth muscle-stimulating substance described by Zucker (16) as present in blood serum and platelets would not be present in our extracts since it is destroyed by boiling in 1 N HCl.

The histamine-like activity present in our extracts may have been originally present, at least partially, in the cells of the nasal secretions; if extracellular, it may have been either free or combined; Anrep et al. (17) have found that histamine is normally excreted in urine in a conjugated and inactive form from which the active base can be released by hydrolysis with HCl. Much more work will have to be done before the significance of this histamine-like activity of nasal secretions can be evaluated.

CONCLUSION

A substance which stimulates guinea-pig ileum is present in extracts of nasal secretions both from "common cold" and allergic rhinitis. The method of treating nasal secretion was designed for the extraction of histamine.

This activity has a dose-response curve similar to that of histamine, and can be measured in the presence of atropine sulfate.

The concentration of this histamine-like substance in nasal secretions varies widely both in the common cold and in allergic rhinitis.

A correlation between the number of eosinophiles and histamine content in nasal secretions was not apparent.

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INFLUENCE OF THE CALCIUM INTAKE LEVEL UPON THE COMPLETE LIFE CYCLE OF THE ALBINO RAT

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Previous papers from this laboratory have recorded the results of calcium enrichments of a basal dietary (diet A, laboratory no. 16) consisting of 1 part by weight of dried whole milk and 5 parts ground whole wheat with table salt and distilled water (Sherman and Campbell, 1935; Van Duyne, Lanford, Toepfer and Sherman, 1941; Campbell, Pearson and Sherman, 1943).

Rat families in our colony are still thriving in the 59th generation on that diet, yet it was found to be improved by enrichment in its calcium content up to a level about three times that of the original (*i.e.*, from 0.19–0.20 to 0.61–0.64 per cent of calcium in the air-dry food mixture).

The present paper records an investigation of the effect of calcium enrichments of a better balanced basal diet richer in protein, riboflavin, and vitamin A as well as in calcium.

Experimental. In the experiments here reported, the basal diet (diet B, laboratory no. 13) consisted of 1 part dried whole milk and 2 parts ground whole wheat with table salt and distilled water. Its average analysis showed 16.0 per cent protein, 0.34 per cent calcium, and 0.5 per cent phosphorus; with an energy value of 4 calories per gram. With this were compared diets 132 and 133 prepared by mixing with the above basal diet enough calcium carbonate to bring the calcium content of these two diets to 0.48 and 0.64 per cent, respectively, without significant change in the percentage of other constituents or in the energy value. These three diets were fed to matched lots and parallel second generation lots of white rats until natural death. Each lot consisted of 3 females and 2 males, the sexes being reared together and allowed to breed without restriction.

Twenty-four such breeding-lots (that is, 72 females and 48 males) were kept throughout life on each of these three diets, one half being animals put on the respective diets at weaning time, the other half being parallel lots of their offspring. The first generation lots were carefully matched at the beginning, each set of three containing litter-mate males and females, the total body weights being the same for each of the three parallel lots. The experimental lots of their offspring consisted of first or second cousins, so started on their respective diets that the parallel lots differed in age by not over ten days at the most. Thus any seasonal influence was excluded from affecting the comparison of the diets.

Other second or third generation animals were selected for study of calcium storage in the body and were killed at 28, 60, 90, 180 and approximately 365 days of age. The results of these studies of the amounts and percentages of body calcium have been reported elsewhere (Lanford, Campbell and Sherman, 1941).

The average records of the animals on these three diets (laboratory nos. 13,

132, and 133) showed no significant differences in growth or adult size, or in the rate of development as indicated by the age at which females bore their first young, or in the average size of the young at weaning. The tabulation of these data is therefore omitted to save space.

Table 1 summarizes the findings on the numbers of young reared per female, the duration of reproductive life, and the lengths of life of the sexes averaged separately.

As none of the differences found (table 1) is more than three times its probable error, and the numbers of experimental animals were fairly large, it may be concluded that, with the favorable basal diet here used, the animals receiving from 0.34 to 0.64 per cent of calcium in their air-dry food were being maintained on a level which under entirely favorable experimental conditions would appear to be a "plateau of optimal nutritional intake." In this connection it is of interest that analyses of representative animals of these same families showed progressively higher percentages of body calcium with increased levels of calcium intake (Lanford, Campbell and Sherman, 1941). That these moderately higher body calcium contents are likely to prove real assets under such vicissitudes as those of human life even if the advantage is not shown in the data of animals so well fed and living such protected lives as these, is a view expressed in personal communications from Dr. P. C. Jeans and from the late Dr. T. Wingate Todd. The animals on higher calcium intake showed somewhat higher blood hemoglobin (Ellis, L. N.: Unpublished work).

TABLE 1

Reproduction records and lengths of life of rats on diets of differing calcium content

	DIET 13, 0.34% Ca	DIET 132, 0.48% Ca	DIET 133, 0.64% Ca
	mean ±*P.E.**	*mean* ±*P.E.*	*mean* ±*P.E.*
Young reared per female, *number*	29.6 ±1.21	32.3 ±1.37	30.0 ±1.31
Duration of reproductive life, *days*	331 ±10.6	355 ±11.5	333 ±11.6
Length of life:			
Males, *days*	706 ±12.2	691 ±13.6	724 ±12.2
Females, *days*	781 ±13.5	839 ±12.8	799 ±16.4

* This precision measure is the classical probable error of the mean.

SUMMARY

The experiments here reported compare the average life histories of 72 female and 48 male rats on each of three diets containing respectively 0.34, 0.48 and 0.64 per cent calcium in the air-dry food mixture. The basal diet was chosen as being as nearly optimal as possible in all other respects. Under this favorable condition, the three levels of calcium intake here studied resulted in essentially equally good records throughout the life histories of the experimental animals. Thus with a diet sufficiently good in other respects, the range of optimal calcium intake extended from 0.34 to at least 0.64 per cent of the air-dry food mixture.

The fact that in other experiments with animals of the same colony, but on a less favorable basal diet, a somewhat higher calcium intake appeared necessary for optimal results may perhaps have an added interest in relation to the long-standing hypothesis that calcium in addition to its specific functions may also exert a general regulatory or stabilizing effect.

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AFFERENT NERVES EXCITED BY INTESTINAL DISTENTION[1]

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In 1934 Herrin and Meek (1) reported that continuous distention of intestinal fistulae in dogs resulted in a condition closely simulating acute bowel obstruction. The dogs showed anorexia, vomiting and other behavior which indicated that they felt sick. There never was any indication of acute pain. These manifestations of intestinal distention did not appear when the fistulous bowel had been denervated by sectioning the nerves in the mesenteric pedicle. This report is concerned with the identification of the afferent nerves excited by distention of the small intestine.

Since then a search in the literature has revealed that in 1908 Cohnheim and Dreyfus (2) reported that intestinal distention in dogs caused vomiting. A rubber balloon was introduced through a duodenal fistula and then filled with 40 to 60 cc. of water. They related that the dog began to masticate, to lick his mouth with his tongue, to make peculiar movements of his head, to breathe deeper and within 1 to $1\frac{1}{2}$ minutes to vomit. Ranson (3) cites the observation of Dmitrenko that inflation of a rubber balloon in a dog's stomach was followed by a rise in blood pressure, a quickening of the pulse and acceleration and deepening of the respiration.

The literature indicates that the vagi and splanchnics are most likely to be involved in these reactions. Suner and Puche (4) distended the stomach of chlorosed dogs with a rubber balloon and observed initially inhibition of respiration, followed by increased amplitude and frequency of movement. Similar results were obtained by distention of the duodenum, jejunum or ileum. Section of the vagi and splanchnics abolished these effects. Morin and Vial (5) distended with a water-filled balloon various segments of small bowel in chlorosed dogs. Distention of the duodenum produced apnea or a decrease in respiratory amplitude, a depression of carotid blood pressure and inhibition of the jejunum. Distention of jejunum resulted in increased rate and depth of breathing and elevation of arterial pressure. Bilateral section of the sympathetic chain in the thorax abolished the effect on respiration and circulation but the reflex inhibition of other parts of the intestine remained. Similar studies have been made recently by Crowley (6). He distended the ileum or jejunum of barbitalized dogs with pressures as great as 150 mm. Hg. The effect upon respiration was the same as that obtained by Suner and Puche and in addition, arterial blood pressure was increased. Vagotomy or vagotomy and adrenalectomy did not abolish the effect. Section of the spinal cord at T 2 and section or cocainization of the splanchnic radicals abolished the effect.

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There is some experimental evidence indicating the nerves concerned in vomiting. Walton, Moore and Graham (7) determined the nervous pathways used in the vomiting of peritonitis which had been produced in cats and dogs by an injection of B. coli. The vomiting was entirely of nervous origin, the impulses being carried over both sympathetic and vagal nerves. Bilateral abdominal sympathectomy, splanchnotomy and intra-thoracic vagotomy were necessary to prevent vomiting. Apparently irritation of the visceral peritoneum is responsible for the vomiting of peritonitis and not the parietal since the various other spinal nerves were intact.

Hatcher and Weiss (8) found that certain emetics, for example mercuric chloride, could cause vomiting by exciting emetic impulses equally well in either the sympathetics or the vagi.

Schrager and Ivy (9) found that distention of the gall bladder and biliary ducts in dogs caused inhibition of respiration, nausea, vomiting and distress. The nausea, vomiting and some respiratory inhibition were abolished by section of the vagi. The distress and some respiratory inhibition were abolished by section of the right splanchnic.

Ashkenaz and Spiegel (10) found that in decerebrate cats, distention of the gall bladder or duodenum or faradic stimulation of the splanchnic nerves evoked a contraction of the panniculus carnosus muscle which resulted in a movement of the skin over the body trunk. This reaction was proven to be a reflex with its afferent path in the splanchnic nerves.

The clinical studies of De Takats (11) indicated that a local block of both splanchnics resulted in anesthesia of the upper abdominal organs. The lower abdomen could be anesthetized by adding to the splanchnic block, the anesthesia of the lumbar rami communicantes.

In addition to the reflexes previously mentioned intestinal distention also excites reflex inhibition of other parts of the gastro-intestinal tract. Youmans (12) has cited the literature and demonstrated that reflex inhibition of other segments of the intestine by distention could be mediated not only through the extrinsic nerves but also through the intrinsic plexi in the bowel wall. However, the latter pathway was less effective.

Methods. With aseptic care Thiry fistulae were made in the first portion of the jejunum in dogs. After complete recovery the fistulous bowel was distended with a balloon as previously described (1). During the course of distention the dog was carefully observed for the initial appearance of anorexia, vomiting and any other indications of a disturbance in his sense of well being. The nervous manifestations usually appeared before any marked disturbance in blood chemistry occurred but if not saline was infused intravenously. After this, the balloon was removed and the dog allowed to recover, after which some one of the denervations was performed.

The vagi were sectioned in the thorax near the diaphragm. The splanchnics were cut as they emerged below the diaphragm. The lumbar chains and ganglia were dissected and excised from the diaphragm to the sacral promontory. After some stage of the denervation was achieved and recovery made, the dog was again tested with distention.

Results. These are presented in table 1 and need little explanation. The dogs vomited and showed anorexia after vagotomy but did not vomit after the splanchnics were cut and the lumbar chains were excised. Therefore, the

TABLE 1

Effect of various denervations upon the response of dogs to intestinal distention

DOG NO.	TYPE OF DENERVATION					
	None	Vagotomy	Splanchnicotomy excision of lumbar chain	Splanchnicotomy vagotomy	Splanchnicotomy vagotomy lumbar chain cut	Splanchnicotomy vagotomy lumbar chain excised
1	Vo. 2 An. 4	Vo. 1 An. 2		An. 4 Vo. 4		
2	Vo. 2 An. 4	Vo. 3 An. 6				Rt. Vo. 2 An 2
		Vo. 2 An. 3				Bi. No symptoms
3		Vo. 2 An 2				
4		Vo. 2 An. 3				
5		An. 2			No symptoms?	
6		Vo. 3 An. 3				
7			An. 7			
8			An. 4			
9	An. 4		An. 6			No symptoms
10	Vo. 1 An. 3		An. 2			
11			Rt. Vo. 6 An. 7			
			Bi. An. 5			No symptoms
12	An. 2		Rt. Vo. 7 An. 7			
			Bi. An. 4			No symptoms
13	Vo. 2 An. 2			Vo. 3 An. 2 Slight		
14	Vo. 1 An. 4				An. 2	No symptoms
15	An. 5				An. 4	No symptoms
16	Vo. 1 An. 8				An. 2 No symptoms	
17						

Vo—vomiting; An—anorexia; Rt.—right; Bi.—bilateral. The number indicates the day of distention on which the disturbance was first observed.

nervous impulses responsible for vomiting during intestinal distention are conducted over the sympathetic nerves and the results obtained on dogs 14, 15 and 16 indicate that only the splanchnics are involved. About the time that the

dogs began to show anorexia and vomiting they also became less active and gave less attention to their surroundings. This together with other variable aspects of their behavior gave one the impression that the dogs were sick. This sick appearance was absent after splanchnicotomy and excision of the lumbar chains even though the dogs still showed anorexia. It might also be pointed out that 3 out of 5 dogs, nos. 14, 15 and 16 seemed to have received disturbing impulses through the lumbar chain into the spinal cord.

From the standpoint of the rôle of distention in the symptomatology of bowel obstruction, it might be pointed out that the vomiting of 7 of these dogs was conclusively proven to be of nervous origin. The dogs vomited with intestinal distention and did not vomit after the proper denervation was attained. This rules out the possibility of any substance from the bowel contents or the wall itself which might cause vomiting by stimulation of the medullary vomiting center.

SUMMARY

Distention of Thiry fistulae in the first portion of the jejunum in otherwise normal, conscious dogs resulted in vomiting and anorexia. The appearance and behavior of the dog at that time gave the observer the impression that the dog was sick.

Vagotomy did not abolish the vomiting or anorexia during intestinal distention.

Bilateral splanchnicotomy and excision of the lumbar chain abolished the vomiting response to distention. Unilateral denervation was not sufficient. The sick impression given by the dog previously was absent although anorexia was present.

Splanchnicotomy, vagotomy and section of the lumbar chains abolished all symptoms due to intestinal distention in 2 dogs but anorexia remained in 3 dogs.

Splanchnicotomy, vagotomy and excision of lumbar chains abolished the anorexia and vomiting when the intestine was distended.

It is concluded that the vomiting excited by intestinal distention is entirely of nervous origin and that the nervous impulses are conducted over the sympathetic nerves. The anorexia present with intestinal distention may be due to impulses from the vagi and also from the sympathetic nerves.

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SOME OBSERVATIONS ON GENERAL SKIN TEMPERATURE RESPONSES TO LOCAL HEATING OF HUMAN SUBJECTS IN A COLD ENVIRONMENT

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The character of the vasomotor responses of the body to local changes in temperature and heat flow has significance in relation to the mechanism of temperature regulation. The effectiveness of heat applications to different body surfaces is also of practical importance in connection with physical therapeutic procedures. In the study reported at this time the effects of prolonged immersion in warm water of parts of the upper and lower extremities have been observed in the normal human. The relative effects of warming in the two situations have been compared, and observations have been made of the duration of the resulting vasomotor response. Certain tentative theoretical deductions will be presented.

Methods. The skin temperature of nine healthy males 18 to 22 years of age were measured by the use of the Hardy and Soderstrom (1) radiation thermometer. Measurements were made with the subjects nude except for shorts and seated on a chair with the feet resting on a rod six inches above the floor. They were kept in a room with temperature, humidity and air movement controlled. The temperature was 16.7 ± 0.5°C. The humidity was 37.5 ± 2.5 per cent and the air flow 25 feet per minute. Measurements of rectal temperature were not made in this group of experiments. Thermocouple measurements on similar subjects in another study under comparable conditions showed that in a cold room at 10°C. while lightly clothed there is an initial rise in rectal temperature followed after about 30 minutes by a return to the initial value.

After a period of 30 minutes in the cold environment the subjects' forearms or legs were immersed for 20 to 25 minutes in water baths controlled thermostatically at 43 to 44°C. and thoroughly stirred. Control studies were made on each subject in which surface temperature measurements were made over 60 to 65 minutes. Each subject was studied in this way at least twice, the total number of control periods being 25, and temperatures were measured over 8 skin areas as follows: 1, dorsum of the left hand; 2, middle lateral left upper arm; 3, left shoulder at tip of acromium; 4, back, over the left scapula; 5, left side, seventh intercostal space between anterior axillary and mammary lines; 6, anterior middle left thigh; 7, posterior middle left calf; 8, dorsal middle left instep. Measurements were made at each of the 8 points in a regular sequence each 5 minutes throughout the course of the experiment. The position of the body changed slightly when the forearms were immersed in the bath. A control study showed no effect on surface temperatures by this alteration. Immersion

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of the arms was to a point just below the elbows and in the case of the legs to just below the knee joints, carefully fixed in each case.

In four experiments referred to separately, temperature measurements were made by skin and rectal thermocouples. In three additional experiments the effects of forearm heating were measured with and without occlusion of blood vessels by application of a pressure cuff above the elbows and inflation to a value just in excess of the systolic pressure.

RESULTS. The skin surface temperatures of the normal young adult male subject fall progressively to varying degrees over the period of one hour in the cold room at 16.7°C. at all skin areas excepting the thigh. The largest fall is 3.3°C. over the instep and the least is 0.8°C. over the scapula. The thigh shows a fall for 50 minutes with a questionably significant rise thereafter. In general, the trunk areas show smaller changes than the extremities, and in the latter the more distal portions show the greater falls in surface temperature. The results of all control measurements have been averaged and plotted in figures 1 to 8 in the solid curves.

Two experimental procedures were employed in these studies; namely, the immersion of either the two forearms or the two legs below the knee in water at 43 to 44°C. for periods of 20 and 25 minutes respectively, after the subject had spent 30 minutes in the cold room. The influence of heating this body surface upon surface temperatures elsewhere on the body was then ascertained. In this way the relative effectiveness upon skin vasomotor reactions of arm and leg surface can be ascertained.

In the case of the areas immersed in either procedure no measurements of surface temperature could be made by the method employed.

When either the forearms or the legs are warmed under the conditions described the surface temperatures over the entire trunk and the thigh and upper arm all tend to rise above the control values and ordinarily above the prestimulation level. Detailed observations in 17 experiments in which the forearms were heated and 21 in which the legs were similarly heated are shown in the figures. In each case the portion of the graph marked "A" shows the results of warming the forearms and "B" the legs. The − or + signs above points indicate the existence or absence of a statistically significant difference between the control and experimental values. To be considered significant a difference was required to be more than three times the probable error of the difference between the means.

It will be noted in figure 1 that when the legs are warmed the dorsum of the hand shows a temperature rise of 2.4°C. The deviation from the control curve is 7 times the probable error at 10 minutes after immersion. Warming the leg is obviously an adequate stimulus for vasodilatation in the hand with the body in the cold environment used. In two experiments not shown in the graph, only one leg was immersed in water at 43°C. There was no significant rise in hand temperature above the control values. Thus, warming one leg was an inadequate stimulus to vasodilatation in the hand, while the same stimulus applied to two legs produced the marked effect seen.

Figure 2 presents the data for upper arm temperatures when the two forearms (A) and the two legs (B) are immersed in 43°C. water. Again it is obvious that local heating produces significant alterations in skin temperature within

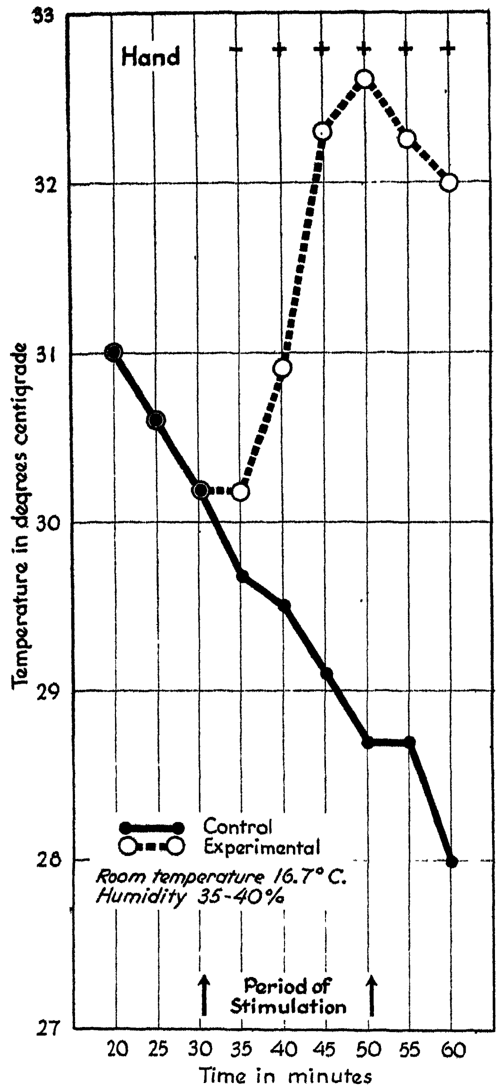

Fig. 1. Surface temperature changes in the dorsum of the left hand in normal subjects in a constant cold environment without and with heating of both legs below the knee by immersion in water at 43–44°C. The — and + signs above comparable points in the two curves indicate absence or presence, respectively, of a significant difference between the control and experimental values.

10 minutes after immersion. There is a significant difference, however, between the effects of immersing the forearms and the legs. The differences between the "experimental" temperatures in cases "A" and "B" in figure 2 at the times

40, 45 and 50 minutes, are statistically significant. Thus warming of the forearms is definitely a more effective measure for upper arm vasodilatation than is the warming of the legs, which have greater surface and mass, as will be discussed more fully later.

The comparative effects of forearm and leg heating upon shoulder surface temperature are seen in figure 3. Here forearm warming produced a significant deviation within five minutes, whereas the earliest statistically significant alteration following leg warming occurred 25 minutes after immersion. The difference between the two "experimental" curves was significant at the 40 minute point.

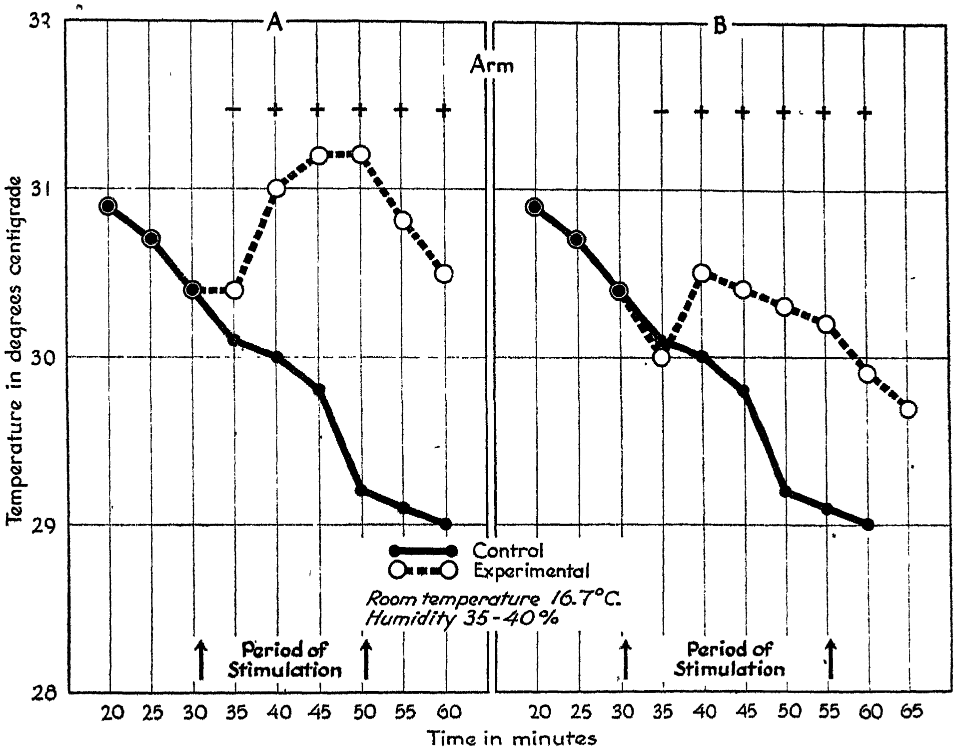

Fig. 2. Surface temperature changes in the upper arm in relation to heating of the forearms (A) or the legs below the knee (B) by immersion in water at 43–44°C.

The responses of the skin over the scapula shown in figure 4 appear to be much smaller. Warming the forearms produced significant changes for only the first 10 minutes, while heating the legs produced a significant change only over the second 10 minute period during the immersion. The differences between the "experimental" curves were significant at the times 50 and 55 minutes. It will be noted that in this case it is the leg heating that produced the more prolonged effects. This is in contrast to the opposite finding in other locations.

The observations on skin temperature on the side of the thorax shown in

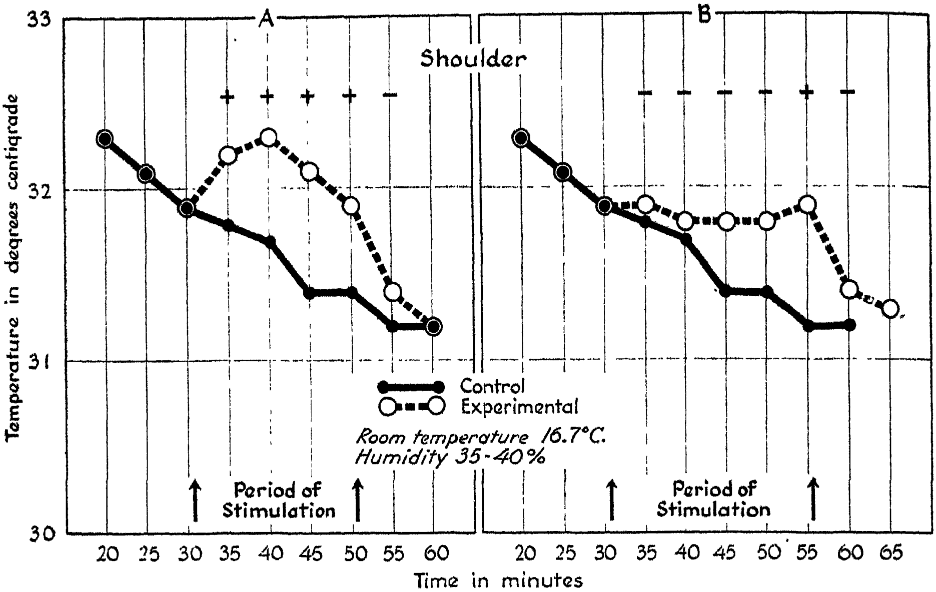

Fig. 3. Surface temperature changes in the left shoulder in relation to heating of the forearm (A) or the legs below the knee (B) by immersion in water at 43–44°C.

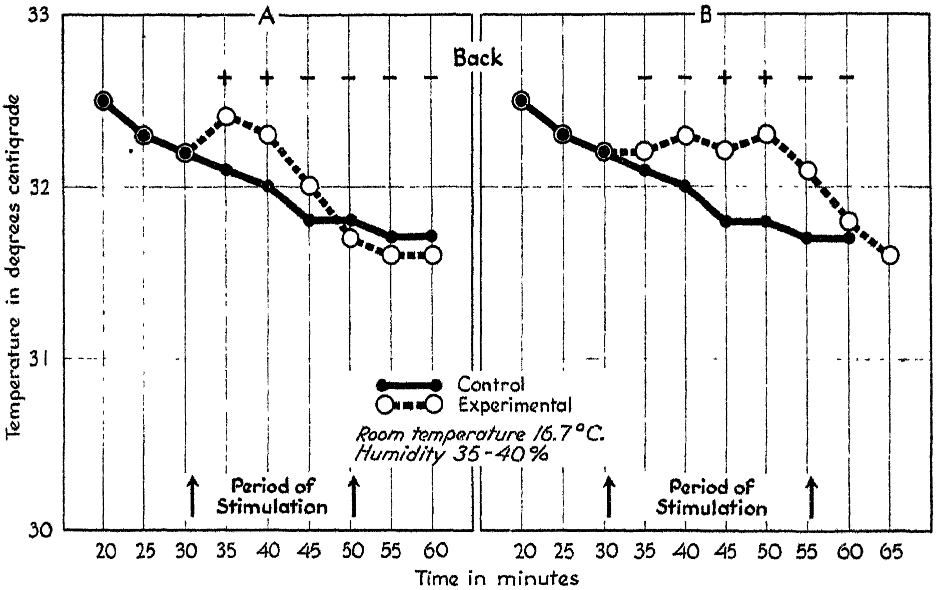

Fig. 4. Surface temperature changes in the back in relation to heating of the forearm (A) or the legs below the knee (B) by immersion in water at 43–44°C.

figure 5 indicate that a significant vasodilatation occurs after 5 minutes of heating of either forearms or legs. However, the effect of warming the legs is evanescent. The differences between the two "experimental" curves are sta-

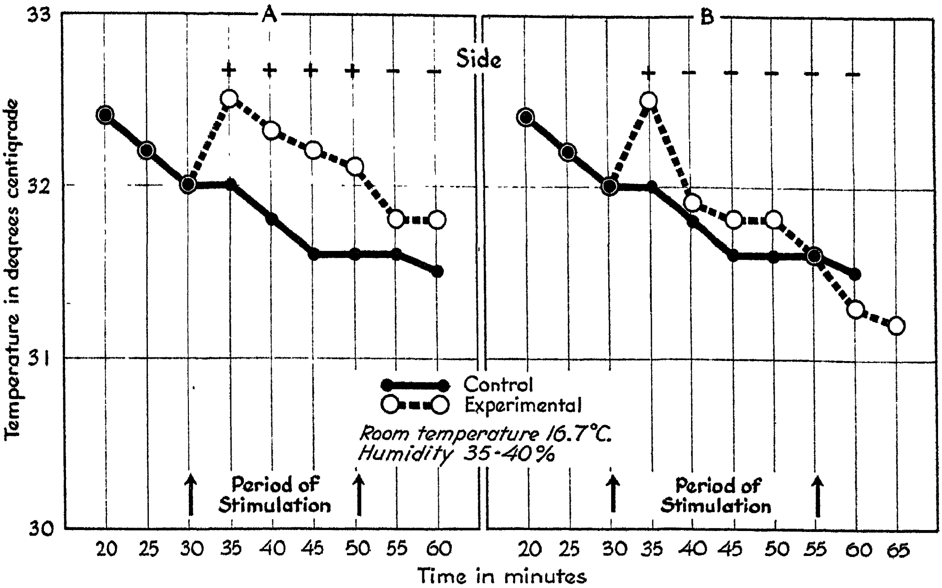

Fig. 5. Surface temperature changes in the left side in relation to heating of the forearm (A) or the legs below the knee (B) by immersion in water at 43-44°C.

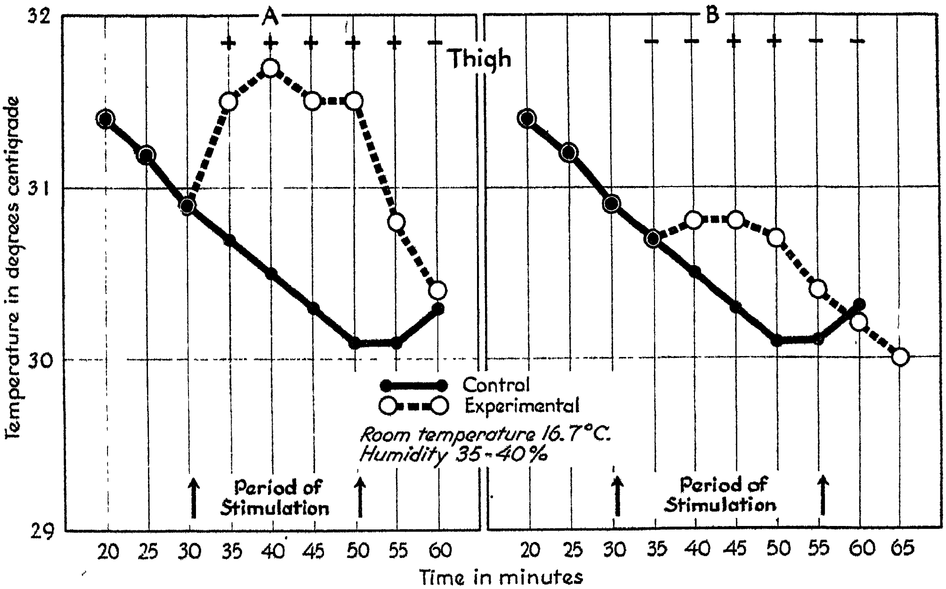

Fig. 6. Surface temperature changes in the left thigh in relation to heating of the forearm (A) or the legs below the knee (B) by immersion in water at 43-44°C.

tistically significant at 40, 45 and 50 minutes. Thus it is obvious that forearm heating is a more effective means of producing skin vasodilatation in the lateral thorax than is leg warming.

When the thigh temperatures are studied in figure 6 an even more pronounced difference between forearm and leg warming is seen. The difference from the control is significant after 5 minutes of immersion for the former and not till 15 minutes with the latter. Furthermore, the magnitudes of the heating

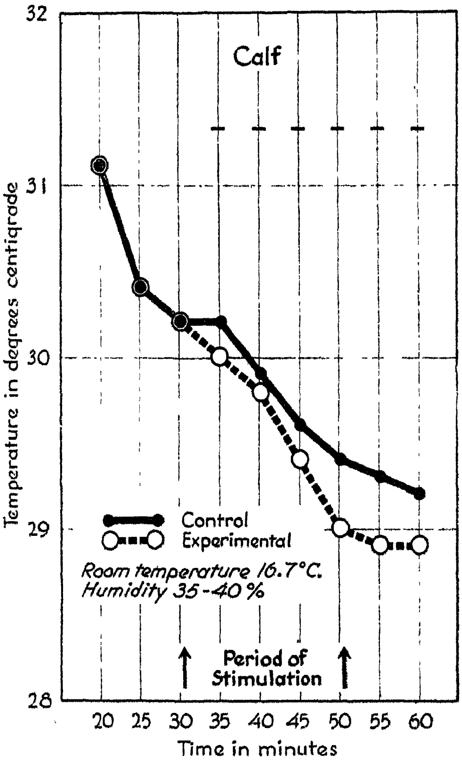

Fig. 7

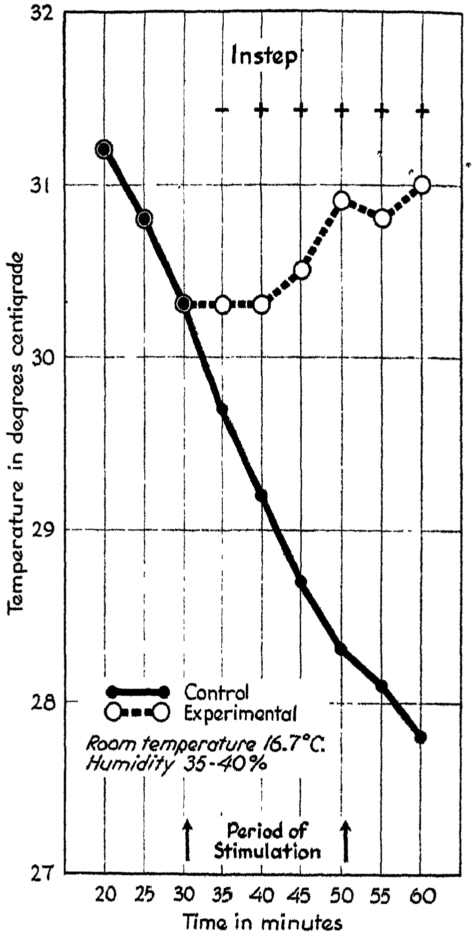

Fig. 8

Fig. 7. Surface temperature changes over the posterior medial left calf in relation to heating the forearms by immersion in water at 43–44°C.

Fig. 8. Surface temperature changes over the dorsal middle left instep in relation to heating the forearms by immersion in water at 43–44°C.

effects are very different and the differences in the two "experimental" curves in "A" and "B" are statistically significant at 35, 40, 45 and 50 minutes, that is, throughout the whole period of forearm heating.

In the case of the calf surface temperatures shown in figure 7 it will be seen that forearm heating produced no significant deviation from the control values. This is in sharp contrast to what is seen in figure 8 for the case of the dorsum of

the foot. Here there is a sharp and significantly large heating effect. The absence of a heating effect upon the calf with large actions on the foot and the thigh indicates that there is not a simple rule relating magnitude of response to more distal parts of extremities.

Four experiments were performed in which measurements were made in two subjects while only one leg was immersed in water at 42 to 44°C. Skin and rectal temperature measurements were made by the thermocouple method in these cases. The skin temperatures in the several areas were not changed from the control values by this experimental procedure. The rectal temperature was not altered by more than 0.2°C. in any case and the average change was a fall of 0.1°C. following immersion in the warm bath. The direction of change, it may be noted, is opposite to that which might have been expected by the application of heat.

No subject felt a subjective generalized sensation of warmth while immersing one leg in warm water while in all of those experiments in which two legs were immersed under otherwise identical conditions such a sensation was reported. It should furthermore be noted that in each of the nine subjects in which forearms and legs were immersed in different experiments there was an invariable report that the subjective sensation of warmth was greater following forearm* than following leg immersion.

In the three experiments in which the two forearms were immersed in water at 43.5°C. with the circulation occluded at the elbow there was, in contrast with the situation observed with a normal blood flow, no significant deviation of the temperature curves from that seen in the controls. Furthermore, although the hands and forearms in the water felt warm there was no generalized subjective sensation of warmth.

Discussion. There is no general agreement as to the mechanism of the generalized peripheral vasodilatation following local heating. Hill (2), Pickering (3) and Gibbon and Landis (4) have shown previously that such vasodilatation in subjects in a cold environment is abolished by vascular occlusion. The studies reported here confirm these observations. It may be concluded that heating of distant skin areas, while the majority of the body surface is cold is not an adequate stimulus to generalized reflex vasodilatation unless there is blood flowing to and from the heated skin. Further, from the fact that heating one leg was ineffective while heating both produced a marked effect, it may be deduced that there is some minimal skin area or total heat input below which heating has no discernible effect on the vasomotor control of the unheated body surface.

The older view of the mechanism of the skin vasomotor response is that local heating produces a reflex vasodilatation as a result of stimulating heat sensitive endings in the skin. That this mechanism operates to some extent cannot be excluded by the present study, nor by those cited above, because a temporary effect of a simple reflex nature could not be detected by skin temperature measurements. Skin temperature reflects blood flow changes only slowly and an evanescent reflex effect might easily be missed.

The mechanism of the effect of the blood flow through the heated part is entirely uncertain. It is not excluded that the cutting off of blood alters the sensitivity of sensory endings in the part. To rule out such an effect completely is impossible on the basis of existing data, but the fact that a feeling of local warmth persists after vascular occlusion argues against its importance. The other possibilities are that return of warm blood to the body proper induces vasodilatation or that some unknown chemical change in the blood depending upon the temperature of the limb from which it flows mediated the effect. The first of these two possibilities is favored by the workers cited. Pickering (3) postulates a temperature-sensitive "central mechanism" responding to temperature changes of 0.01°C. to 0.04°C. in the tissues. However, Uprus, Gaylor and Carmichael (5) find that vasodilatation may occur on local warming while the rectal temperature is steadily falling as much as 0.3°C. in an hour. Furthermore, in our subjects, warming, inadequate to produce the demonstrable vasodilatation, nevertheless was associated with a slight fall in rectal temperature which is in contrast to the tendency to rise in the cold room when heat is not applied. Pickering (3) says concerning the central mechanism: "Its responsiveness suggests that the temperature sensitive mechanism is a specialized receptor or receptors. It is probably not the temperature center itself".... Since the central body temperature as measured in the rectum may vary oppositely to the direction expected we suggest the possibility that sensitive endings located in certain veins may perhaps mediate this effect. Studies on this question are projected.

The observations reported at this time demonstrate that heating the legs below the knee is less effective in producing generalized skin vasodilatation than is identical heating of the forearm and hands. A single exception is the response seen in the skin of the back where the reverse was noted. The usual finding is of interest because the surface area warmed is considerably greater in the former than in the latter case. Measurements by planimeter of a paper mould fitted carefully to all of the skin surfaces of a typical subject showed the total skin area for the forearm and hand to be 1000.2 $cm.^2$, and 1451.4 $cm.^2$ for the leg and foot. The latter is thus 45 per cent greater than the former, while the magnitude of the heating effect was in the reverse ratio.

The frequency of distribution of heat sensitive endings in the skin is, however, much less in the leg and foot than in the forearm and hand (Goldscheider, 6). However, it seems unlikely in view of the abolition of vasodilatation by vascular occlusion that this difference accounts for the effects observed.

The rate of blood flow through the upper and lower extremities may determine the effect seen. Per unit of volume the blood flow through the forearm and hand is greater than through the leg and foot according to Abramson and Fierst (7). The difference diminishes with increasing temperature but appears to remain significant at 43°C., according to Abramson, Katzenstein and Ferris (8). There are insufficient data available to ascertain whether the greater effect of forearm than of leg warming reported here is actually in proportion to the blood flow difference, but it appears possible that it constitutes a major factor.

The fact that continuous warming of two lower legs or forearms produces in most situations only a temporary rise in skin temperature indicates that for some reason the stimulus loses its effectiveness with time. This fact appears to mean that the temperature sensitive mechanism may react more vigorously to changes in temperature than to the absolute value. The rôle of adaptation in this phenomenon has not been investigated. Moreover, it must be recalled that the temperature sensitive endings in the skin may mediate reflex changes in a given local segment without being able to compete with a central mechanism in controlling general skin vasomotor reactions. Thus there may be and probably is a central synthesis of influences from several sources ultimately determining the vasomotor state in any given skin area. The effect of local cold competes with the effect of central heating in determining the response. In this way a decreasingly effective central stimulus could result in the results seen.

SUMMARY

1. Studies are reported of skin temperature measurements with nine normal male subjects exposed to local heating while maintained in a constant environment at a cold temperature.
2. Local heating by immersion in water at 43 to 44°C. produces a larger rise in skin temperature in all locations except the back when the two forearms are immersed than when the legs, up to the knee, are immersed.
3. Immersion of one leg alone does not produce a significant general skin temperature rise under these conditions.
4. Immersion of both forearms with circulation occluded by pressure cuffs above the elbow results in no sustained rise in general skin temperature. The sensation of warmth in the forearms and hands is retained in spite of vascular occlusion.
5. The subjective feeling of general bodily warmth is proportional to the general skin temperature rise.
6. Except for the feet and the hands the skin temperature rise following local heating is temporary in spite of continuous application.
7. The rectal temperature remains constant within 0.2°C. in subjects in the cold room at 16.7°C. with or without application of local heat. There is a tendency for rectal temperature to fall slightly upon local heating.
8. Assuming that skin temperature changes reflect alterations in blood flow through the surface tissues it is deduced that prolonged vasodilatation due to adequate local heating is probably mediated by some type of central mechanism. This is in agreement with earlier suggestions of the same sort. However, it is not believed possible that general body temperature can determine the effect because vasodilatation can occur with a falling rectal temperature.

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CENTRIPETAL REGENERATION OF THE 8TH CRANIAL NERVE ROOT WITH SYSTEMATIC RESTORATION OF VESTIBULAR REFLEXES

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A selective recovery of function has been found to follow centripetal regeneration of the optic nerve after its complete transection in amphibians. The ingrowing optic axons make their way into the visual centers of the brain and there re-establish functional reflex associations in a systematic, predetermined manner such that the differential spatial values of the various retinal points are restored in their original form (4–9). The regulatory growth factors responsible for this orderly formation of differential reflex relations in the brain centers are presumably similar in nature to those by which the inherent patterns of intricate synaptic inter-relationships are formed originally and prefunctionally in ontogenetic development. Little is known of the actual governing mechanisms, however, and further evidence is needed regarding the systematic development of synaptic associations both in nerve regeneration and in ontogeny.

The present experiments were undertaken to find out if the 8th cranial nerve root after complete transection in the adult is capable of regenerating axon connections to its proper nuclei in the medulla and, if so, whether the diverse functional properties of the various vestibular fibers, correlated with their central reflex relations, are restored at random or with systematic selectivity as in the case of the optic system. The 8th nerve happens to be well suited for testing selectivity in recovery following regeneration because it contains fibers to a number of separate end organs, the differential reflex properties of several of which are readily determined by simple functional tests. In the frog the end organs supplied by the 8th nerve include the cristae of each of the 3 semicircular canals and the maculae of the utriculus and the sacculus, the lagena, the pars neglecta, and the pars basilaris (1). There is thus enough functional variety among the 8th nerve fibers to render negligible the possibility that the regenerating axons could by chance alone establish suitable central connections in sufficient numbers to restore proper function. Any consistent restoration of normal discrete reflex associations for the different end organs, therefore, must clearly indicate discriminatory, selective factors in the recovery process.

METHODS. In 20 tree frogs (Hyla squirella) bilateral section of the 7th and 8th cranial nerve roots was performed intracranially through an incision in the roof of the pharynx. The roots of the 7th nerve were divided along with those of the 8th because the two enter the medulla together. This did not detract from but rather enhanced the significance of the experiments as far as positive results were concerned, because the presence of the extra regenerating fibers lessened further any opportunities for fortuitous re-establishment of correct

central connections. With fine jewelers' forceps the nerve roots were pinched off and pulled and teased apart until all fascicles of the 7th and 8th nerve root complex had been severed, after which the frayed nerve ends were roughly approximated. Eight control cases also were prepared in which the 7th and 8th nerve roots were widely excised and the auditory capsules broken to prevent any recovery. General operative technic and after care of the animals were the same as in previous experiments (4–7).

Tests to detect loss and return of the various reflex functions mediated by the 8th nerve were made at 3 to 5 day intervals after operation. Observations were made separately of the effects of angular acceleration or deceleration of the animals in both directions in the 3 planes determined by the 3 primary axes of the body. Also noted were the effects of linear acceleration in different directions, righting reactions from an inverted position both in and out of water, assumption of postural attitudes on different inclines, leaping, climbing, swimming, other general activities and arousal responses to auditory stimuli. At the end of 3½ months the animals' heads were fixed in Bouin's solution, sectioned at 10 mu in different planes and prepared for microscopic examination by the Bodian activated protargol method.

RESULTS. *Immediate effects of nerve section.* The immediate effects of operation in both experimental and control groups were similar and involved the usual profound loss of equilibration consistent with numerous descriptions in the literature. The following brief account is sufficient for the present purposes. Righting reactions, particularly those performed in water, were severely impaired. The animals were unable to swim without somersaulting or rolling and their inco-ordinated struggles terminated as often in the upside down position as right side up. Leaps through the air also were badly directed and resulted in somersaulting and rolling in mid air with the animals commonly landing on their backs or sides. Instead of clinging fast in normal manner to vertical objects, they frequently fell off when climbing or alighting upon them. The head often assumed and was allowed to remain in various odd postures. All reflexes to angular acceleration in all directions in all three primary planes were abolished except for the optokinetic response which, being slower and weaker and present when there is no acceleration, is readily distinguished from the vestibular reactions. The residual visual adjustments disappeared after transection of the optic nerves in 3 cases so tested. Exaggerated abrupt movements of the head and body along with forced circling reactions were also exhibited but had subsided after a few days and hence were probably irritative rather than deficiency phenomena. There was no indication that the 8th nerve root had escaped complete transection in any of the animals.

Extent of recovery. About 1½ months after operation, the animals were all blinded by section of the optic nerves. This made it possible to estimate more easily and accurately the final degrees of recovery by quieting the animals and by eliminating all visual equilibratory actions that might otherwise be confused with vestibular function.

In the control group 2 animals died shortly after operation but the other 6

survived in fair condition. These controls showed no recovery of the principal test reactions. There seemed to be some slight improvement in their posture and ability to move about and remain in an upright position and to cling to the substrate, but even this may have been in part a result of the subsidence of irritative phenomena. The amount of recovery achieved in the various test performances by the controls over the 3½ month observation period was practically negligible, in any case, and for purposes of comparison with the experimental group, the state exhibited by the controls is designated as one of zero recovery.

On the 21st day after operation 3 of the experimental group showed signs of recovery of vestibular responses to horizontal angular acceleration. By the 26th day these and 4 additional cases were displaying unmistakable responses of the vestibular type on the horizontal turntable. Thereafter, recovery continued to make noticeable progress in the experimental group as a whole over a period of about 3 weeks. Five of the 20 experimental cases attained complete recovery by this time in that they came to exhibit responses closely approaching normal in all of the different tests. Two cases, on the other hand, never showed any recovery at all and another one died during the second week after operation. The remaining 12 of the experimental group achieved various intermediate degrees of recovery ranging from nearly complete recovery in most tests to almost no recovery.

Selective character of recovery. In those animals which showed complete recovery, the reflex reactions elicited by different patterns of labyrinthine stimulation were not randomly confused, disorderly, or massive in nature as if the fibers from the different end organs had made central connections in a fortuitous manner. On the contrary, discrete reactions of normal quality were evoked consistently by different types of stimulation. Horizontal angular acceleration to the right caused compensatory horizontal turning of the head to the left, while conversely, deceleration caused an opposite movement to the right. Corresponding responses in the opposite directions were produced by horizontal spin to the left. Tipping the animals on their longitudinal axis to the right or to the left caused in each case typical compensatory tilting movement of the head in the proper direction to tend to keep the head on a horizontal plane. Tipping the animals head-downward on the transverse body axis resulted in a counteracting elevation of the head just as in the normal animal and, vice versa, tilting the body head-upward caused an active depression of the head. Thus these various types of labyrinthine stimulation elicited discretely each its own particular appropriate response.

Righting from an inverted position was again performed with proficiency indistinguishable from normal. The animals recovered their ability to swim in a straight line correct side up, to make long leaps through the air without landing upside down, and to alight and climb on vertical walls without falling off. Normal postures were regularly assumed and in all general activities behavior of apparently normal quality was displayed.

Some attempt was made to test functional recovery of the auditory as well

as vestibular fibers of the 8th nerve in these animals. The method used was to see if the animals could be aroused from their characteristic daytime repose to a state of alertness by means of auditory stimuli. It was found that tearing, rustling, crushing, or scratching of paper (noises with high overtones) would elicit arousal reactions with reliable consistency in 3 of these 5 cases showing complete recovery. The arousal response consisted of elevation of the eyeballs, withdrawal of the nictitating membranes, dilatation of the pupils, increase of respiratory rate, and sometimes raising of the head or even movement of the whole body. The lack of consistency in the responses of the other 2 of the 5 experimental cases tested does not necessarily indicate that the hearing of these 2 was abnormally poor, for there was comparable individual variation in tendency to respond among a group of 7 normal animals. Although the positive responses seemed convincing and were not made by any of the control cases, this evidence must be accepted with caution because of the impossibility of ruling out completely other avenues of stimulation such as convection currents in the air, sympathetic vibration of the substrate, or other uncontrolled factors. These arousal reactions were abolished by severance of the 8th nerve root in 5 cases so tested (2 experimental, 3 normal), but this might have been a secondary result of the severe effects of 8th nerve section.

In the cases showing intermediate degrees of recovery, as well, the recovered labyrinthine reflexes were of normal character. Functional restoration was defective in these cases only in the lack or weakness of responses, and not in the mode of response; i.e., the defects appeared to be basically quantitative, not qualitive. For example, an animal might show fair or good responses in the correct direction to angular acceleration in the horizontal plane, while at the same time reactions to tilting in the vertical plane would be absent or extremely weak. In 5 cases recovery was definitely asymmetrical with respect to the two sides so that the animals tended to lean or turn predominantly to one side. But the various test responses superimposed on these postural biases were made in the correct direction. Only one exception to the foregoing statements was noted. This was a case in which for a period of over a week during recovery, rapid lateral tilting caused the animal to lean farther off balance rather than to compensate in the normal manner. These reversed responses, however, are apparently characteristic of frogs in which only the utriculus remains functional (11). They cannot, therefore, be considered a sign of atypical central reflex associations and more probably signify that recovery of function of the end organs other than the utriculus was tardy and deficient. In summary the only defects in the recovered functions were such as to indicate that they arose not from abnormal functional associations but rather from absence of function.

Judging by the number of different kinds of sensory end organs supplied by the 7th and 8th nerves, at least 11 distinct classes of afferent fibers had regenerated from the point of transection with ample opportunity among them for a completely chaotic interspersion into abnormal pathways and with very little chance for particular fiber types to be directed by mechanical factors alone to their proper central terminations. And yet in recovery there was no reliable sign of any abnormal central associations while it was quite clear on the other

hand that in all cases showing recovery different classes of vestibular fibers had managed consistently to re-establish their own proper type of reflex associations. The conclusion is evident that restoration of reflex relationships in the medulla had not been fortuitous but had been systematically regulated in a prearranged order.

Further checks and controls. That the observed recovery of vestibular reflexes was a product of 8th nerve regeneration and not ascribable to compensatory action of other sensory systems was clear from the following: *a*, the striking difference between those cases which showed complete recovery and those, including the controls, which showed no recovery at all; *b*, lack of deterioration of the principal test reflexes following optic nerve section; and finally, *c*, abolishment of all the recovered reactions by resection of the regenerated 7th and 8th nerve roots. The conclusion that functional abnormalities where they occurred were due to a lack of recovery and not to a positive recovery of atypical central associations was supported further by the histology. The severed nerve roots were found to have undergone a dense regeneration in those cases in which recovery of function was good while in those cases in which functional recovery had been lacking or poor, nerve regeneration proved to be absent or very slight. In many cases there was a severe reduction in the number of cells in the 8th nerve ganglion and it is very possible that lack of complete recovery was caused in large part by direct or secondary damage to the nerve ganglion itself which lies close to the point of transection. An additional but less important factor appeared to be the escape and misdirection of regenerating fibers outside the dural sheath of the medulla. In the region of transection there was considerable intermixing and rearrangement of fibers and fiber bundles.

Attempts to determine the regulatory factors. The experimental results thus far show that the 8th nerve root is capable of regenerating centripetally to re-establish functional associations within the central nervous system and that the resultant restoration of vestibular reflexes is achieved in an orderly systematic manner. They raise the additional problem, however, of whether the orderliness of functional recovery is attained directly by growth mechanisms alone or whether it involves functional readjustment processes of some type such as learning. The following observations and few additional experiments conducted mostly on late larval stages are concerned with this further problem.

The following indicated that the learning process is not involved in regulating recovery. *a*. The recovered reflexes were found to survive decerebration plus optic nerve section in both the adult (*H. squirella*, 4 cases) and in the tadpole (*R. grylio*, 5 cases). *b*. There was no indication of a practice period in recovery. The first time that a response could be elicited by angular acceleration, for example, it was made in the correct direction. *c*. The vestibular system is highly reflex in nature and relatively unsubject to reintegration by learning. Even in mammals no direct connections with the cortex or a cerebral cortical center have been found (3). Finally, *d*, what is known of the learning capacity of amphibians in comparable situations indicates that it is far too limited to achieve readjustments of this sort (8).

The inference that no type of functional regulation whatever is involved in

the recovery is suggested by the following: Those vestibular reflex movements of the eyeball which on the occurrence of imposed head movements tend to maintain a steady view of the visual field are pronounced in the tadpole and readily demonstrated by tilting the animals in water either in an open dish or in a close-fitting test tube. It was found that these reflexes were restored in their proper form by regeneration of the 8th nerve root in the tadpole (*R. grylio*, 4 cases; *R. clamitans*, 2 cases) even though the animals had been blinded by previous excision of the optic nerve. Blinding presumably eliminated the functional value of these eyeball responses and yet in spite of this the vestibular eye movements were systematically re-established in their regular pattern. This evidence against functional adaptation, though suggestive, is not crucial because the eye movements may conceivably be part of a larger motor adjustment, other components of which retained a regulative functional value.

An attempt was made to set up a more critical test of the rôle of functional regulation by reversing the orientation of the vestibular sense organs. Recovery of normal responses under such conditions would indicate functional readaptation whereas recovery of responses in reverse would indicate that the re-establishment of reflex relations is predetermined by growth factors regardless of functional adaptation. Homoplastic transplantation of the left labyrinth into the site of the excised right labyrinth with reversal of the antero-posterior axis was carried out in a range of late larval stages of *R. grylio* and *R. clamitans* (16 cases), but none of these animals showed any signs of functional recovery although kept as long as 3 months after operation. Even in premotile embryonic stages such transplants or even direct reimplantations have previously been found to fail functionally, although apparently good nerve connections with the medulla are formed (10). Possibly a developmental stage or condition will eventually be found in which the inverted labyrinth will establish functional central nervous connections and thus provide a conclusive answer to the above question.

SUMMARY AND CONCLUSIONS

Fibers of the 8th nerve root after its complete transection in 17 adult and in 11 larval anuran amphibians succeeded in regenerating across the nerve gap, into the central nerve stump and into the medulla where they re-established functional reflex connections. Furthermore, despite inexact apposition of the nerve ends and distortion of the intraneural fiber pattern in the region of severance, the different fiber types of the vestibular root succeeded in regaining their original innate modes of function in an orderly selective manner. The normal character of the recovered reflexes indicated that those nerve fibers connected peripherally to the crista of the horizontal semi-circular canal formed their own type of central reflex relations distinctly different from those formed by neighboring fibers innervating other end organs of the labyrinth. Similarly the fibers of the anterior and posterior canals systematically re-established in each case their own distinct and appropriate type of central associations. As far as could be determined from the tests made, the fibers of all the various end organs of the labyrinth succeeded in recovering selectively their original proper type of central reflex relations.

Just how this systematic recovery of specific functional associations was accomplished remains unknown. That the precision of recovery can be attributed simply to indifferent mechanical guidance of the regenerating fibers is excluded by the manner in which the nerves were broken and apposed as well as by the microscopic appearance of the nerve scar. General knowledge of nerve regeneration (13) moreover indicates that such an interpretation is quite out of the question and may be disregarded. That the ingrowing fibers terminated in a random manner in the centers and these random anatomical associations were later readjusted to function adaptively by a "conditioning" or learning process, may also be excluded for reasons already enumerated above.

Two possible alternative interpretations remain open. First, function may conceivably have influenced the formation of synaptic terminals in some such manner as presumed in the theory of neurobiotaxis (2). It has often been supposed, particularly with regard to the development of reflex patterns in ontogeny, that the adequacy of functional effects may somehow have an organizing influence on the growth of synaptic associations. Some evidence counterindicating any functional regulation in the present cases is cited above but it is admittedly incomplete. Secondly, it is possible that the systematic re-establishment of central associations was predetermined essentially by non-functional factors such as physico-chemical axon specificities and selective contact affinities between the different axon types and neurons of the vestibular centers. This latter interpretation is strongly favored by other available evidence regarding both the establishment of basic integration patterns in ontogeny (12) and the re-establishment of central synaptic associations in nerve regeneration (5–8). The required constitutional specification of the vestibular nerve fibers could conceivably arise embryologically through induction effects of end organ differentiation in the labyrinth.

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EFFECTS OF THYROID FEEDING, THYROIDECTOMY AND ADRENALECTOMY ON THIOUREA INTOXICATION IN RATS

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The action of thiourea in inducing pulmonary edema and pleural effusion in old rats presents a complex problem in physiology. This phenomenon, first emphasized by Richter and Clisby (1), has also been studied by Mackenzie and Mackenzie (2), by Griesbach, Kennedy and Purves (3) and by Dieke and Richter (4).

In the experiments to be reported the effect of 50 mgm. of thiourea in 2 cc. of water injected intraperitoneally was studied on groups of thyroid fed, thyroidectomized and adrenalectomized male and female rats. The youngest rats used were approximately three months and the oldest more than a year old. The principal data are summarized in the following table.

TABLE 1

Effect of thyroid feeding, thyroidectomy and adrenalectomy on thiourea intoxication in rats

GROUPS	NO. OF RATS	AGE	NO. SHOWING DEFINITE SYMPTOMS	NO. DIED	AVERAGE DURATION OF LIFE	AVERAGE PLEURAL FLUID
		mo.			*hrs.*	*cc.*
Thyroid fed males	6	6	6	6	3.2	5.3
Control males	3	6	3	3	6.0	10.5
Thyroid fed males	6	3	6	6	4.8	6.8
Control males	6	3	6	3	8.6	7.9
Thyroid fed females	6	6	6	5	5.0	5.2
Control females	3	6	3	3	9.0	9.0
Thyroid fed females	6	3	6	5	6.7	5.9
Control females	6	3	0	0		
Thyroidectomized males	7	6	4	0	*	8.0
Control males	6	6	6	3	6.0	10.5
Thyroidectomized females	8	6	1	0		
Control females	6	6	6	3	9.0	9.0
Adrenalectomized males	5	12	2	2	8	8.0
Control males	10	12	7	2	6	8.5
Adrenalectomized females	7	12	0	0		
Control females	8	12	0	0		

* Sacrificed after 5 hours.

1. *Effect of thyroid feeding.* These rats were given 75 mgm. of desiccated thyroid containing 0.3 per cent organic iodine daily for six days mixed with a dry food ration[1] of 8 grams per rat, moistened with milk, and tested with thiourea on the seventh day. Of twelve males, all died in convulsions.[2] The six months old rats survived, on the average, 3.2 hours and the three months old rats, 4.8 hours. Of the nine controls of the same litters, six died while three of the three months old rats recovered, although they had dyspnea. Of the twelve thyroid fed females, ten died and the remaining two had dyspnea. Of the nine litter mate control females, only the three older rats died, while the six three months old rats had no evident dyspnea. The males were thus more susceptible to thiourea intoxication as determined by the number of surviving rats, the survival time, the severity of the symptoms and the amount of fluid recovered from the pleural cavity.

2. *Effect of thyroidectomy.* Of the seven thyroidectomized males, none died although four had moderate dyspnea. Two with the severest symptoms were sacrificed at the end of 5 hours and they had an average of 8 cc. of fluid in the pleural cavity. Of the six control males, all had severe dyspnea and three died. Of the eight thyroidectomized females, none died and only one was dyspneic, while of the six control females three died and the remaining three were moderately dyspneic. The thyroidectomy and control groups were of the same age, about six months. Thus thyroidectomy had a definite protective effect in each sex, but it was much more striking in the females, just as the females were found to be less susceptible to thiourea intoxication in the case of the thyroid fed intact rats. Of the thyroidectomized females that showed no definite symptoms, four were sacrificed after six to seven hours, one had 2.5 cc. of fluid in the pleural cavity, while the other three had no excess fluid. This protective effect of thyroidectomy is at variance with the report by Mackenzie and Mackenzie (2) on two thyroidectomized rats and also with the observations of Griesbach, Kennedy and Purves (3) who concluded that the toxicity of thiourea "is unrelated to thyroid activity."

3. *Effect of adrenalectomy.* All the rats used in this experiment were about a year old. Both adrenals were removed at one operation and the animals were given 0.5 per cent NaCl in the drinking water. Of the five males, two died and there was an average of 7 cc. of fluid in the pleural cavity. The remaining three did not show any definite symptoms. Of ten control males, two died and

[1] Rolled oats, 1 part; hominy, 4 parts; meat scrap, 1 part; dried milk, 0.6 part; supplemented with dried yeast and salt.

[2] These convulsions are terminal and are believed to be due to asphyxia secondary to pulmonary edema and pleural effusion. This is accompanied by cyanosis, emphysematous deformity of the chest and the rapid onset of general rigor mortis (within 2 min. after death). While pulmonary congestion, edema and pleural effusion are the most prominent anatomical findings, we have regularly noted congestion and edema of pancreas and to a lesser degree of the mesentery and retroperitoneal tissue. This edema of the abdominal structures was more prominent in the thyroid fed rats although because of their short survival time the volume of pleural exudate was less than in rats surviving for longer periods.

five others had severe symptoms but survived. Of the seven female adrenalectomized rats, none showed any definite symptoms, nor did any of the eight control females. Again the males were more affected.

The result in the adrenalectomy experiment was surprising in view of the well established lowered resistance or hypersusceptibility of adrenalectomized animals to so many physical and chemical agents and indicated that the lack of adrenal cortex function was not an essential factor in the susceptibility to thiourea intoxication.

DISCUSSION. We have confirmed the observation (1, 2) that old rats are more susceptible than younger rats to thiourea intoxication. Thyroid feeding in our experiments eliminated the age difference. However, the youngest rats used were three months old. Thyroid feeding also caused a striking increase in thiourea intoxication as determined by the percentage of rats that died, the rapidity of onset and the severity of symptoms and the duration of life. The mechanism of this effect is unknown. The observation of Griesbach, Kennedy and Purves (3) that 1.3 mgm. KI administered subcutaneously for four days protected both thyroidectomized and intact rats from thiourea intoxication shows that it must act, as they have indicated, in some way other than through the thyroid gland.

Thyroidectomy under our conditions afforded a striking protection against thiourea intoxication. All of the fifteen thyroidectomized rats survived, while six of the twelve controls died. This is in sharp contrast with the thyroid feeding experiment where twenty-two of the twenty-four thyroid fed rats died, whereas only half of the eighteen litter mate controls died. One must conclude that the thyroid hormone plays an important rôle in the intoxication. Our experiments showed a sex difference in susceptibility. This difference is not mentioned in the literature. It was present in all groups—thyroid fed, thyroidectomy and adrenalectomy, but was partly masked in the thyroid fed rats as was also the age difference. We have no explanation of this sex difference at present but this phase is being investigated. The fact that adrenalectomy does not increase the incidence of thiourea intoxication merely means that the lowered resistance due to loss of cortical function is not an important factor. However, the possible rôle of epinephrine in this phenomenon is not excluded. Indeed, the slight protection noted in the few adrenalectomy experiments might be interpreted as due in part to loss of epinephrine while, on the other hand, it is possible that the increased susceptibility of male rats in all groups of experiments might be related to the larger amount of chromaffin tissue which they possess.

CONCLUSIONS

1. Thiourea intoxication is influenced by age and sex.
2. Thyroid feeding greatly increases the incidence, severity and rapidity of onset of symptoms and death in both sexes.
3. Thyroidectomy decreases the incidence of thiourea intoxication.
4. Adrenalectomy does not increase the incidence of thiourea intoxication.

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